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HUMAN PAPILLOMAVIRUS AND SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

Whole scientific world is talking about Human Papillomavirus (HPV) and his role in the oncogenesis, but after all who is this virus? .HPV is small (50-55 nm in diameter), non-enveloped, double-stranded DNA virus which primarily infect epithelial cells of the skin and mucous membranes. They are associated with a variety of clinical manifestations that range from benign lesions to cancer. The spectrum of diseases associated with HPV infections has accompanied humans throughout evolution. Papillomatous and verrucous skin and genital lesions have been described since ancient Greece and Rome. But until early twentieth century, research of HPV has not been started yet. Indication that now recognized HPV-associated diseases could be related to sexual transmitted infection dates from 1842 year when Italian physician Rigoni-Stern noted a high frequency of cervical cancer in married women, widows and prostitutes, but their rare occurrence in virgins and nuns. After visualization of papillomavirus particles in human warts by electron microscopy and characterization of HPV genome interest for HPV started to arise. Zur Hausen postulated and analyzed a possible role of HPV in cervical cancer development. The first HPV types (HPV16 and 18) were isolated from cancer biopsies of the cervix and were cloned in 1983 and 1984, respectively. Further epidemiological studies have confirmed that cervical infection by high-risk (HR) HPV types is the main risk factor for cervical cancer development. The role of HPV in the pathogene-

sis of squamous cell carcinoma of head and neck (SSCHNs) was first described in 1983, when histopathological features consistent with HPV infection were identified in oral cancers. Two years later, viral DNA from high-risk HPV-16 was detected in an oral carcinoma by Southern blot hybridization. By the early 1990s, discoveries of HPV in association with oropharyngeal carcinomas became consistent. In 2008, the Nobel Prize for Medicine was awarded to Dr. Zur Hausen for the discovery of the infection etiology of cervical cancer.

Currently, we have more molecular evidence that indicates a relationship between high-risk HPV and the pathogenesis of oropharyngeal cancers. Expression of HPV *e6/e7* oncogenes are the current criterion standard for determining a causal role of oncogenic HPV in human tumors. The *E6* and *E7* genes produce oncoproteins and are responsible for the degradation of p53, which are important events that lead to dysregulation of the cell cycle and thereby immortalization of the cells. The silencing of p16 by promoter methylation is commonly observed in SCCHNs. In contrast, HPV is readily detected in HPV-positive tumors and is highly correlated with p16 expression, which is most likely the result of transcriptionally active HPV infection. Therefore, there is a relationship between HPV the expression of p53 and p16 in oropharyngeal cancer and clinicopathological variables. The diagnosis of HPV positivity should enter as a routine test for these patients. The most clinically

useful test, *in situ* hybridization, is available in only few centers, so that is unfortunate, even if p16 immunohistochemistry may serve as a reasonable surrogate and PCR – molecular detection of HPV DNA. It is widely accepted that the most common risk factors for SCCHNs are tobacco use, heavy alcohol consumption, betel quid chewing, and high-risk HPV infection. High-risk HPVs are currently well accepted as causative agents for most cervical cancers and have recently been associated with a subset of head and neck cancers. Oropharyngeal cancer has emerged as another mucosal cancer that is associated with HPV infection. HPV DNA is present in 20% of all SSCHNs and in nearly 60% of tonsillar cancers. Traditionally, most cases of oropharyngeal cancer were associated with chronic tobacco and alcohol use. This exposure resulted in the mutation loss of the genes p16 and p5. Decreased use of tobacco coupled with an increase in the HPV + of oropharyngeal cancer has shifted the majority of these types of cancers to now occur more commonly in non-smokers. An “epidemic “ of HPV oropharyngeal related cancer has been identified and attributed to HPV + disease in many cases thought to result from oral genital contact.

Patients with HPV + cancer are less likely to smoke tobacco and less likely to use alcohol heavily, are having a better dentition, are having a higher incidence of oral sex practice, younger and higher socioeconomic status. Having in mind this two types of oropharyngeal cancers, HPV+ and HPV – and their molecular changes that concern the expression of p16 and p53 genes , we can conclude that patients with HPV + cancer have an higher expression of p16 and a lower expression of p53, responded well to treatment and showed

good prognoses. The T stage in TNM (tumour, node, and metastasis) stage does not differ from HPV status, HPV + tumors are more likely than HPV –tumors to have a lower T stage, whereas advanced T stage is a significant risk factor for recurrence and death, independently of HPV status. Patients with p16 + oropharyngeal tumors are less likely to have persistent viable tumor in neck lymph nodes after (chemo) radiotherapy and hypothesized that a post treatment planned neck dissection may perhaps be avoided. Several authors indicate that the impact of HPV status must be evaluated in prospective clinical trials and included as a stratification factor to avoid potential confusion .The current American Joint Cancer Committee staging system for oropharyngeal cancer needs to be modified in order to be better reflect different prognoses for patients with HPV+ or HPV – tumors and those with vegetating or infiltrating tumors.

HPV-related SCCHN represents a new entity well- defined with a well- known characteristic that includes young age, good performance status, male gender, nonsmoking and nondrinking status, basaloid tumor histology and high-risk sexual behavior. The practicing oncologist needs to be aware of these new findings, and HPV testing with PCR or FISH, should be perform routinely. For now, results will have significant prognostic though not therapeutic implications. Still, changes come rapidly in this field and we expect that different treatment will be available to these patients in the near future.

In conclusion, what do we wish for these patients? We wish a modified TNM stage for HPV + tumors? We wish a genic and antiviral HPV specific – therapy? We wish a fuller utilization of HPV vaccine

and public education measures?

Personally, I am having great expectations within the scientific progress in this field so we could have some day the responses to those questions above, all for an early diag-

nosis, a specific treatment and a long term prognosis for the HPV positive related SCCHN s. Multidisciplinary dedicated clinical practice with integrated research, I consider to be an important aim.

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REFERENCES

1. Al-Swiahb N. Jamil, Chien Chih-Yen, Prognostic Impact of p16, p53, Epidermal Growth Factor Receptor, and Human Papillomavirus in Oropharyngeal Cancer in a Betel Nut–Chewing Area, *Arch Otolaryngol Head Neck Surg*. 2010; 136(5): 502-508.
2. Chien CY, Su CY, Fang FM et al. Lower prevalence but favorable survival for human papillomavirus-related squamous cell carcinoma of tonsil in Taiwan. *Oral Oncol*. 2008;44(2): 174- 179.
3. Chien CY Huang, Cheng JT, Chen CM, Hwang CF, Su CY .The clinic pathological significance of p53 and p21 expression in squamous cell carcinoma of hypopharyngeal cancer. *Cancer Lett* 2003; 201 (2): 217- 223.
4. Ciardiello F, Tortora G . A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res* 2001;7(10): 2958- 2970.
5. Fakhry C, Gillison M L. Clinical implications of human papillomavirus in head and neck cancers. *J Clin Oncol* 2006;24(17): 2606- 2616 .
6. Gillison ML. Human papilloma virus-related diseases: oropharynx cancers and potential implications for adolescent HPV vaccination. *J Adolesc Health* 2008; 43(4) (suppl): S52- S60
7. Gillison ML, Koch WM, Capone RB et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000; 92(9): 709- 720. Klusmann JP, Weissenborn SJ, Wieland U et al. Prevalence, distribution, and viral load of human papillomavirus 16 DNA in tonsillar carcinomas. *Cancer* 2001; 92(11): 2875- 2884.
8. Reimers N, Kasper HU, Weissenborn SJ et al. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. *Int J Cancer* 2007;120(8): 1731- 1738.
9. Sok JC, Grandis JR Genetic screening for oral human papillomavirus infections and cancers of the head and neck. *Clin Cancer Res* 2008;14(21): 6723- 6724 .
10. Whiteside MA, Siegel EM , Unger ER . Human papillomavirus and molecular considerations for cancer risk. *Cancer* 2008; 113(10) (suppl): 2981- 2994.
11. Zur Hausen H (2009). Papillomaviruses in the causation of human cancers – a brief historical account. *Virology* 384: 260-265.

ADHD (ATTENTION DEFICIT HYPERACTIVITY DISORDER) – A TROUBLING ENTITY, SOMETIMES PERPETUATING DURING ADULT LIFE

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ADHD (ATTENTION DEFICIT HYPERACTIVITY DISORDER) – A TROUBLING ENTITY, SOMETIMES PERPETUATING DURING ADULT LIFE (Abstract): Attention deficit hyperactivity disorder (ADHD) is considered a neurologic development disorder resulting in impairment of attention and inhibitory control, manifested as attention deficit, hyperactivity, impulsiveness; symptoms should develop between age six and twelve and have to persist for more than six months. Approximately 30-50 % of the diagnosed cases are manifesting the disorder during adulthood and 2.5-5 % of the adults are suffering of ADHD. Genetics are important factors in ADHD, being involved in 75 % of the cases, as well in the persistence of ADHD during adult life. Three subtypes of ADHD are described – one in which is predominating the attention deficit, one with predominant hyperactivity and impulsiveness and a third combined subtype. Diagnosis criteria in ADHD are established by the American Psychiatric Association (DSM criteria) and by World Health Organization. Differential diagnosis is mainly considering bipolar disorder and borderline personality disorder. Management of ADHD is including behavioral therapies and medication, alone or combined. Stimulant medications such as amphetamine represent the therapy of choice, being effective in 80 % of the cases. New data are underlying the need for following up of the cases during adulthood, since the risk for development of psychiatric conditions such as depression, anxiety, as well as the suicidal behavior is higher than in the general population. **Keywords:** ADHD/ATTENTION DEFICIT HYPERACTIVITY DISORDER, HYPERACTIVITY, IMPULSIVENESS.

DEFINITION, GENERAL FEATURES

Attention deficit hyperactivity disorder (or hyperkinetic disorder) is considered a neurologic developmental disorder in which are present problems of executive functions, like attention control and inhibitory control which are generating attention deficits, hyperactivity or inappropriate

impulsiveness (related to age) (1,2).

Diagnosis is established only when symptoms are lasting for more than six months and are appearing between age six to twelve.

In 2013 World Health Organization estimated 39 million people affected by the disorder; 3-5 % of children are affected. ADHD is three times more frequent in boys

ADHD (Attention Deficit Hyperactivity Disorder) – a troubling entity, sometimes perpetuating during adult life

than in girls. Around 30-50 % of people diagnosed during childhood show symptoms as adults and 2.5-5 % of the adults have the disorder (3).

The disorder was first clearly described by George Still in 1902. Others terms used for ADHD are minimal brain dysfunction or minimal brain damage (5).

ADHD is divided into three subtypes: a form with predominant attention deficit, one with predominant hyperactive-impulsive behavior and one combined (4).

MAIN ETIOLOGIC AND PATHOPHYSIOLOGIC FEATURES

Even if the cause of ADHD is not known, the disorder is believed to be the result of interactions between genetic and environmental factors (5). Twins studies demonstrated that the disorder is often inherited, genetics being involved in 75 % of the cases. Genetic factors seem to be also involved in the persistence of ADHD during adulthood. Genes involved are mainly regulating dopamine neurotransmission (DAT, DRD₄, DRD₅, TAAR₁, MAOA, COMT and DBH) (6,7).

From the evolutionary perspective, hyperactivity should have been beneficial in situation of risk exposure, like exploring new areas, thus should have been beneficial for the community even if harmful for the individual (8).

A reduction in the volume of certain brain structures was observed in children with ADHD, as well as a proportionally important decrease in the volume of the left-sided prefrontal cortex. Posterior parietal cortex is also thinner in ADHD subjects (9).

Recent models of ADHD are demonstrating that the disease is associated with dysfunction in central neurotransmitter

systems, especially those involving dopamine and norepinephrine. Dopamine and norepinephrine pathways which are projecting into the prefrontal cortex and striatum are responsible for the modulation of the executive functions (cognitive control of behavior), motivation, reward perception and motor function; these pathways play an important role in the pathophysiology of ADHD (10).

DIAGNOSIS

Diagnosis is established on person's childhood behavior and mental development, excluding effects of drugs, medications or psychiatric conditions, which otherwise may explain the symptoms. It may be regarded as the extreme of one or more common human traits. The diagnosis is not excluded or confirmed by response to specific medications. Imaging studies of the brain are not relevant for diagnostic purposes (11).

ADHD is classified among disruptive behavior disorders, along with oppositional defiant disorder, conduct disorder and antisocial personality disorder (12).

According to the American Psychiatric Association (DSM criteria) are differentiated three subtypes of ADHD: one subtype is mostly characterized by inattentiveness (easily distracted, forgetful, daydreamer, with concentration and completing tasks difficulties). The predominant hyperactive-impulsive type is manifesting as restless, hyperactive, with difficulties in waiting and remaining seated, immature conduct; the third type is a combination of the first two subtypes, or the combined type (13).

Inattention symptoms include: being easily distracted, missing details, switching frequently from one activity to another; difficulty in maintaining focused on one

task; not seeming to listen when spoken to; daydreaming, getting easily confused and moving slowly, showing difficulties in processing information as quickly and accurately as others; difficulty in focusing attention on organizing and completing a task or learning new data; becoming bored with a task after a few minutes (13).

Hyperactivity is characterized by fidget and squirm, talking continuously, trouble to sit during dinner, school or doing homework; the subject is all the time in motion and has difficulties in achieving quiet activities. These symptoms of hyperactivity tend to fade away with age and in teens and adults with ADHD they become an inner restlessness (14).

Impulsivity is revealed by symptoms such as: impatience, inappropriate comments, acting with no regard for consequences, difficulty in waiting (as their turns in games) and often interrupting others talking or activities.

Diagnosis is established in the presence of six to nine long term symptoms of inattention, hyperactivity-impulsivity or both, lasting for at least six months (15).

Symptoms should appear at the age of six to twelve, in more than one situation, like for example at home and at school, and they must cause social, educational or work related problems (14).

Differential diagnosis must take into consideration the bipolar disorder and borderline personality disorder. Symptoms of anxiety disorder, antisocial personality disorder and mental retardation may sometimes overlap with ADHD or may occur at the same time with ADHD. Diseases which symptoms may mimic ADHD are especially hyperthyroidism, seizure disorder, lead intoxication, sleep apnea and drug interactions (16). Primary sleep troubles may

cause attention deficit, while ADHD may alter sleep quality; sleep problems must be treated in children with ADHD.

THERAPEUTIC OPTIONS

Management of ADHD is including behavioral therapies and medication, alone or in combination.

Behavioral therapies are recommended mainly in those cases with mild symptoms and in preschool aged. They include behavior therapy, cognitive behavioral therapy, interpersonal therapy, family therapy, school-based interventions, neuro-feedback (12).

An important factor in reducing psychological problems (such as major depression, school failure, criminality, drug use disorders) is the friendship with persons who are not involved in delinquent activities (17, 18).

Stimulant medications such as amphetamine are the therapy of choice being effective in approximately 80 % of cases.

Alternatives include bupropionum, guanfacine, clonidine, atomoxetine. Stimulant medication is also improving the academic performance (19).

Overdose of stimulants is associated with symptoms of mania; very rare (0.1 %) such symptoms may appear at therapeutic doses in the first weeks of treatment with amphetamine or methyl-phenidate. Use of stimulants in doses above the therapeutic range is associated with addiction and dependence (18,19).

PROGNOSIS

Proportion of children showing criteria for ADHD drop at half in the three years following the diagnosis, whether if treated or not. ADHD is persisting into adulthood in approximately 30-50 % of cases.

ADHD (Attention Deficit Hyperactivity Disorder) – a troubling entity, sometimes perpetuating during adult life

Generally, ADHD individuals are developing coping mechanism as they become mature, thus attempting to compensate their symptoms. However, higher risk for depression, anxiety and suicidal attempt is stressing the importance of surveillance and appropriate interventions in adults with ADHD (11, 16).

Controversies in what concerns ADHD are ranging from the opinion that ADHD should be viewed as situated in the normal range of behavior to viewing it as a genetic condition. Most specialists are considering it a medical condition, at least in the cases with severe symptoms (20).

CONCLUSIONS

In what concerns ADHD diagnosis, we notice that while the mild symptoms do not need to be diagnosed, most severe cases must be treated and followed-up since the disorder is interfering with social and educational activities, as well as with work in these subjects.

Recent data demonstrated that persistence of ADHD during adulthood (2.5-5 % of the adults) is increasing the risk for development of psychiatric conditions (depression, anxiety, suicidal behavior) which recommend the surveillance and adequate therapy in these cases.

REFERENCES

1. Diamond A. Executive Functions. *Ann.Rev. of Psychol* .2013; 64: 135-168.
2. Childress AC, Berry SA. Pharmacotherapy of attention-deficit hyperactivity disorder in adolescents. *Drugs* 2012; 72(3): 309-325.
3. Willcutt EG. The prevalence of DSM-IV attention-deficit/hyperactivity disorder. A meta-analytic review. *Neurotherap* 2012; 9(3): 490-499.
4. Coleman WL. Social competence and friendship formation in adolescents with attention-deficit/hyperactivity disorder (ADHD). *Adolesc.Med State Art Rev* 2008; 19(2): 278-299.
5. Thapar A, Cooper M, Eyre O, Langley K. What have we learnt about the causes of ADHD ? *JChild Psychol Psychiatry* 2013; 54(1): 3-16.
6. Franke B, Faraones V, Asherson P, Buitelaar J et al. The genetics of attention deficit/hyperactivity disorder in adults; a review. *Mol Psychiatry* 2012; 17(10): 960-987.
7. Kebir O, Tabbane K, Sengupta S, Joobar R. Candidate genes and neuropsychological phenotypes in children with ADHD: review of association studies. *J Psichiatry Neurosci* 2009; 34(2): 88-101.
8. Glover V. Annual Research Review: Prenatal stress and the origins of psychopathology: an evolutionary perspective. *J Child Psychol Psychiatry* 2011; 52(4): 356-367.
9. Cortese S, Kelly C, Chabernaud C, Proal E et al. Toward systems neuroscience of ADHD: a meta-analysis of 55 fMRI studies. *Am J Psychiatry* 2012; 169 (10): 1038-1055.
10. Fusar-Poli P, Rubia K, Rossi G, Sartori G, Balottin U. Striatal dopamine transporter alterations in ADHD: pathophysiology or adaptation to psychostimulants ? A meta-analysis. *Am J Psychiatry* 2012; 169 (3): 264-272.
11. Gentile JP, Atiq R, Gillig PM. Adult ADHD : Diagnosis, Differential Diagnosis, and Medication Management. *Psychiatry* (Edgmont) 2006; 3(8): 25-30.
12. Wolraich M, Brown M, Brown RT, Du Paul G, Earls M et al. ADHD: clinical practice guideline for the diagnosis, evaluation and treatment of attention-deficit/hyperactivity disorder in children and adolescents. *Pediatrics* 2011; 128(5): 1007-1022.
13. American Psychiatric Association 2013. *Diagnostic and Statistical Manual of Mental Disorder* 5th ed. Arlington: American Psychiatric Publishing, 2013, 59-65.

14. Steinau S. Diagnostic Criteria in Attention Deficit Hyperactivity Disorder – Changes in DSM5. *Front Psychiatry* 2013; 4:49-50.
15. Lambek R, Tannock R, Dalsgaard S, Frillingsgaard A, Dann D. Validating neuropsychological subtypes of ADHD: how do children with and without an executive function deficit differ? *J Child Psychol Psychiatry* 2010; 51(8): 895-904.
16. Show M, Hodgkins P, Caci H, Young S, Kahle J et al. A systematic review and analysis of long-term outcomes in attention deficit hyperactivity disorder: effects of treatment and non-treatment. *BMC Med* 2012; 10:99: 1186-1196.
17. Fabiano GA, Pelham WE, Coles EK, Guagy EM. A meta-analysis of behavioral treatments for attention-deficit/hyperactivity disorder. *Clin Psychol Rev* 2009; 29(2): 129-140.
18. Bellani M, Moretti A, Perlini C, Brambilla P. Language disturbances in ADHD. *Epidemiol Psychiatry Sci* 2008 ; 20(4) : 311-315.
19. Pliszka S. practice parameter for the assessment and treatment of children and adolescents with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 2007; 46(7): 894-911.
20. Segal MM. We cannot say whether attention deficit hyperactivity disorder exists, but we can find its molecular mechanisms. *Pediatr Neurol* 2014; 51(1): 15-16.

NEWS

TIMING OF ESOPHAGECTOMY IN MULTIMODAL THERAPY OF ESOPHAGEAL CANCER

Neoadjuvant radiochemotherapy [n(R)CT] has become the standard of care in the multimodal therapy concept for patients with locally advanced esophageal cancer. The question is whether the length of the interval between completion of n(R)CT and surgery can affect the postoperative outcome, tumor response and long-term survival. In a study of 106 patients with adenocarcinoma and squamous cell carcinoma of the esophagus, treated between 2006 and 2013, on the basis of the median time interval to surgery, patients were divided into two groups [group A ≤ 40 days (n = 54) and group B > 40 days (n = 52)] and compared concerning demographic data, preoperative risk scores, morbidity, outcome, tumor response and long-term survival. Group A showed a trend towards a higher mortality risk as preoperatively assessed by the physiological and operative severity score for the enumeration of mortality and morbidity in esophagogastric surgery patients (O-POSSUM) (p = 0.064) and group B showed a trend towards a higher rate of complete responders (p = 0.097). So, concerning perioperative morbidity and mortality, delayed surgery after n(R)CT shows no benefit for the patient's outcome; however, the rate of complete tumor response is higher in patients with a time interval of more than 40 days, although this does not influence long-term survival or recurrence rates. (Müller A.-K. et al. Timing of esophagectomy in multimodal therapy of esophageal cancer : Impact of time interval between neoadjuvant therapy and surgery on outcome and response. *Der Chirurg* 09.2015, doi: 10.1007/s00104-014-2916-6. 86 : 9 : 874-880).

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ETHICAL AND GENETIC ASPECTS REGARDING PRESYMPTOMATIC TESTING FOR NEURODEGENERATIVE DISEASES

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ETHICAL AND GENETIC ASPECTS REGARDING PRESYMPTOMATIC TESTING FOR NEURODEGENERATIVE DISEASES (Abstract) Neurodegenerative diseases, such as Alzheimer's dementia, Huntington's chorea, Parkinson's disease or spinocerebellar ataxia, manifests into adulthood with an insidious onset, slowly of progressive symptoms. All of these diseases are characterized by presymptomatic stages that preceded with many years of clinical debut. In Parkinson's disease, more than half of the dopaminergic neurons of the black substance are lost before the advent of motor characteristic manifestations. In Huntington's chorea, the progressive neurodegenerative disease could be diagnose prenatal and presymptomatic by analyse of the number of CAG repeats in exon 1 of the huntingtin gene. A similar mechanism represented by expansion of trinucleotide repeats during hereditary transmission from parents to children was identified in fragile X syndrome, spinocerebellar ataxia, spinal muscular and bulbar atrophy, or myotonic dystrophy. Presymptomatic diagnosis in all these progressive diseases raise many ethical issues, due to the psychological impact that can cause the prediction of a disease for which there is currently no curative treatment. Therefore, a positive result can produce serious psychological trauma and major changes in the lifestyle of the individual, instead, a negative result can bring joy and tranquillity. But the problem arises if presymptomatic testing in these neurodegenerative diseases brings greater benefits compared to the possible psychological damage, which can add the risk of stigmatization or discrimination. **Keywords:** NEURODEGENERATION, DYNAMIC MUTATION, PRESYMPTOMATIC TESTING, ETHICS ASPECTS.

Neurodegenerative diseases (ND) are hereditary or sporadic conditions characterized by progressive nervous system dysfunction, often associated with atrophy of central or peripheral nervous system structures. They are a heterogeneous group of age-associated, chronic illnesses of varying aetiologies, the main challenges for

healthcare systems, including informal care and long-term care facilities, in the coming decades (1).

ND had a bad prognosis, after a period of 7–12 years of progressive deterioration of multiple areas of brain, including memory, reasoning, communication and the skills needed to carry out daily activities (2).

The recent discoveries of disease-causing genes in neurodegenerative diseases have generated considerable interest and debate for both physicians and patients regarding routine genetic testing of some ND in the clinic. Molecular genetics provide a powerful tool in the diagnosis of many neurodegenerative diseases and can help better define and classify many of the heterogeneous inherited neurodegenerative disorders. Genetic testing could allow early confirmation of diagnosis and appropriate institution of genetic counselling, but also may provide a genotype-phenotype correlation, select specific patients for clinical drug trials, and offer a better understanding of pathogenesis of the disease (3). Pre-symptomatic testing is usually performed at risk-population and investigate a person with a clear-cut family history, who has no symptoms of ND at the time of testing (4) and looking for genetic mutations that have a high penetrance, such as homozygous or compound heterozygous mutations in recessively inherited genes (e.g. *PINK1* and *DJ1* genes, in Parkinson's disease) or dominantly inherited mutations (like *SNCA* or *LRKK2* genes, in Parkinson's disease) (5).

Various positions have been advanced regarding the issue of predictive genetic testing for unpreventable or untreatable adult-onset disorders (6). On the basis of a medical benefit argumentation, some have argued that the absence of measures to prevent the disease or its complications or to treat the disease is a reason not to perform this test (7). Although still acknowledging the importance of medical benefit as a justification for predictive genetic testing, it has been recognized that in exceptional circumstances to apply the predictive test could be better than wait that child will arrive in adulthood (8).

Often, the communication of genetic information is a difficult issue and may have a powerful psychosocial impact on the child and its parents. Thus, in the case of a positive test, children could present anxiety or depression, and the parents could present a feeling of guilty. This process could be complicated by several factors. Usually, genetic testing is made only one time during the life and could have important consequences. Thus, the individual needs to think very carefully before deciding to take such a test. In majority of diseases is still not an ideal biomarker able to improve differential diagnosis, track disease progression and measure treatment efficacy and the method could have not conclusive results or be genetically uninformative (7, 8).

However, the feasibility of routine genetic testing of ND in the clinic is still fraught with many unanswered questions. "Which gene must be analysed in a particular case?" "Which clinical features are more adequate to select patients for genetic testing?" "Is the sensitivity and specificity of the genetic test high enough?" "Which are the consequences of a positive or ambiguous result?" "Which will be the patient's and physician's attitudes in case we will discover a genetic abnormality?" (6).

Genetics of neurodegenerative diseases *Alzheimer's disease*

Alzheimer's disease (AD) is the most prevalent ND, accounting 50-70 % of all dementias. In a report of Commission of the European Communities, from 2009 is estimated that 7,300,000 peoples from Europe present AD and probably in 2020 the number will be double (9). The pathogenic mechanisms imply structural and chemical changes in the brain, leading to the death of neurones. For late-onset AD

cases the mechanism implies interaction of multiple genetic and environmental factors. Early-onset (before age 65) AD tends to cluster within families, but represents only 10% of AD (10). Among these, one half is caused by a heritable mutation in gene encoding one of 3 proteins: presenilin-1 (PS1), presenilin -2 (PS2), or amyloid precursor protein (APP). All these mutations are dominant, and the carriers develop AD in nearly 100% of cases. For multifactorial forms the most important gene seems to be the gene of apolipoprotein E (APOE). The gene is located on chromosome 19 and presents three variants: APOE ϵ 2, APOE ϵ 3 and APOE ϵ 4. Only APOE ϵ 4 has been validated, by genetic linkages, as an AD risk factor. The heterozygous for APOE ϵ 4 have a 3-5 time higher risk, while homozygous for APOE ϵ 4 have a 8-18 time higher risk for late-onset AD. However, a genetic test for APOE ϵ 4 has little predictive value (11, 12).

Other genes which are linked to increased risk of AD are *CLU*, *PICALM*, *CR1*, *BIN1*, *ABCA7*, *MS4A*, *CD33*, *EPHA1* and *CD2AP*. Variants of these genes are linked to significant differences in risk of Alzheimer's, but their effects are much smaller than for APOE (10-13).

Despite the progresses concerning genetic, behavioural, cardiovascular, and nutritional risk factors, long-term prevention studies have yet to be conducted to see if the incidence of AD can be reduced.

Parkinson disease

Parkinson disease (PD) is characterized by a loss of dopaminergic cells in the *substantia nigra pars compacta* and the presence of Lewy bodies (14). The recent new discoveries of a number of disease-causing genes (such as α -synuclein, parkin, *UCHL1*, *PINK1*, *DJ-1*, *LRRK2*) in PD have

generated considerable interest for presymptomatic genetic testing of PD in the clinic. Early-onset autosomal-recessive PD forms were associated with mutation in *PINK1* and *DJ-1* (*PARK7*) genes (15, 16). *LRRK2* (*PARK8*) gene is implied in autosomal-dominant PD with similar phenotype to sporadic PD, but the brain changes generated by *LRRK2* mutations are extremely variable. The most common mutation of *LRRK2* gene is G2019S substitution present in different populations worldwide. Other possible target genes are *PRSS25*, *UCHL1*, synphilin and *NURR1* (17). However, the promises and limitations of genetic testing in PD must be confirmed by detailed scientific studies, which will allow also guidelines and recommendations for such genetic testing.

Huntington disease

Huntington disease (HD) is a neurodegenerative, autosomal dominant disorder with late-onset, caused by the expansion of a CAG repeat in the first exon of the HD gene, located on chromosome 4p16.3. Usually symptoms begin in 30s or 40s decades by involuntary choreic movements, loss of voluntary control of movement, followed by cognitive impairment and personality disorder tending to depression, anger and temper outbursts (18).

The number of CAG repeats is correlated with clinic manifestation of the disease. Normal individuals have 6 – 26 CAGs (class 1) and the allele is stable. In the presence of 27–35 CAGs (class 2) phenotype is normal, but the allele is expandable during spermatogenesis. The individuals with 36–39 CAGs (class 3) have a phenotype with reduced penetrance. The presence of more than 40 CAGs (class 4) is associated with fully penetrated forms (19, 20).

The test for the HD gene is fast, rela-

tively inexpensive, and highly sensitive, with positive and negative predictive values approaching 99 percent. Genetic testing can be performed at any stage of development, even before the onset of symptoms. Many ethical aspects could be identified in the predictive testing of HD, like autonomy, beneficence, non-maleficence, inequality, paternalism or even medical errors. An example of ethical issue could be the case of monozygotic twins, when one brother would to apply the predictive test and other brother do not accept this. In this case is an ethical conflict between autonomy and coercion, but also is necessary to quantify which action is more beneficial: to know if mutation is present or to not know is the mutation is present (21).

Spinocerebellar ataxia diseases

Spinocerebellar ataxias (SCAs) constitute a large group of heterogeneous disorders characterized by a degeneration of the spinal cord and the cerebellum, that cause imbalance, poor coordination, and speech difficulties. Some types of ataxia are inherited, including dominantly inherited diseases and about 30 different gene mutations have been found. Among the more common inherited ataxias are *Friedreich's ataxia* and *Machado-Joseph disease*. Ataxia can also be acquired after stroke, multiple sclerosis, tumours, alcoholism, peripheral neuropathy, metabolic disorders, and vitamin deficiencies. One of the common genetic defects is an expansion of a CAG triplet repeat in genes that regulate the production of different proteins, but the number of CAG repeats varies among the different types of SCAs. Normal individuals with premutation have an increased risk of developing symptoms late in life and also to have abnormal children (22, 23). The correlation between the mutations and the disor-

ders remains unclear, but one explanation may be that the encoded abnormal protein has a toxic effect on neurones, which are successively destroyed. Molecular genetic testing for CAG repeat length is a highly specific and sensitive diagnostic test (24). If the mutation is known in the family, prenatal diagnosis and pre-implantation diagnosis (PGD) are possible. Genetic testing is available for spinocerebellar ataxia types 1, 2, 3, 5, 6, 7, 8, 10, 12, 13, 14, 17, 28; dentate-rubro-pallidoluysian atrophy (DRPLA); ataxia-telangiectasia (A-T); Friedreich's ataxia (FRDA); Ataxia with oculomotor apraxia types 1 and 2; Marinesco-Sjogren Syndrome; Ataxia with Vitamin E deficiency; Fragile X associated tremor/ataxia syndrome (FXTAS); mitochondrial recessive ataxia syndrome (MIRAS); autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS); and Episodic ataxia types 1 and 2 (20, 22-26).

Particularities of genetic testing for a neurodegenerative disease?

In hereditary forms of ND genetic testing can be applied before or after the setup of symptoms. However, the test does not predict the age of setup and the evolution of disease. The major reason for undergoing testing is to find out for the sake of one's children, or for family planning. However, the use of this test for the minor persons is denied, and the only exception is the presence of symptoms before the age of 18 (27).

Most clinical genetic units follow recommended guidelines or programmes, as the waiting process and dealing with the result are very stressful situations, both for the person tested and for the family. The guidelines indicate the need of several meetings with a multidisciplinary team

(that include a geneticist, a neurologist, a psychiatrist, an ethical counsellor) started by a preliminary meeting when the data from familial history is stored. After this meeting, follow a pretesting meeting (when are discussed the beneficial and the disadvantages of testing) and finally a meeting when are presented the results and are discussed the consequences (positive or negative) of genetic analyse. In the present, the diagnoses of ND are frequently made on clinical grounds, sometimes supported by radiological methods. The application of genetic testing for ND should be reserved to patients who have a family history of ND or those with very young age at onset (28-30).

Ethical aspects of genetic testing for adult-onset disorders and ethical aspects

Testing of young people for adult-onset disorders by presymptomatic and predictive genetic testing is the most controversial predictive testing because we have not any treatment that could prevent the onset and progression of the disease (30).

A big problem concerning presymptomatic genetic testing in high risk individuals for ND is related to answer to question - *Do people really want to know the truth?*. Other difficulties are related to collect DNA samples from relatives, who need neurological examination and genetic counselling, problems that raise complex psychological, ethical, legal and moral conundrums (31).

Presymptomatic genetic testing for neurodegenerative diseases generates some ethical conflicts. The authors identified four types of issues. First, in some ND exists juvenile form (for example HD) and this could complicate the decision to make or not the test. Second: in some diseases

the preventive measures or the therapies are uncertain. Third: the best interest is interpretable; in some situation the best interest for child is not identical with best interest for family and in these situations the physicians must protect the rights of child in relation with family. And finally, the guidelines must be concordant with local legal or cultural context (32).

Most people have these tests in hopes of negative results, but inevitably, such hopes are often dashed by tests that come up positive. Many other subjects (in particular, those in families with a history of disease) choose to be tested so that, in the event of a positive test, they can make lifestyle changes or pursue clinical interventions that may alter the course of the disease to which they are predisposed. Unfortunately, a positive genetic testing for ND do not allow a preventive or curative treatment like in other diseases (i.e. breast cancer, colorectal cancer) and this could generate psychological trauma. Thus, a positive DNA test for HD or (early-onset) AD means that, if the person lives to middle or late middle age, the disease *will* manifest, and will be unstoppable once it does. This explains why in generally only about 15-25 percent of people at risk get tested (33).

For some ND, the pathogenic mechanism is linked to a variable amplification of DNA repeats sequences during vertical transmission and generates the phenomenon of anticipation. Also, the severity of the symptoms is correlated with the size of the amplification, but it is difficult to predict severity in a future generation based on mutation size in a parent (34).

Other problem that complicates the genetic testing in ND is the varied penetrance. For example, the penetrance of G2019S mutation associated with Parkin-

son's disease is highly age dependent, increasing from 17% at age 50 years to 85% at age 70 years (35).

So, in the absence of a possible clinical response, a test confirming or even suggesting future development of a fatal neurodegenerative condition can have a devastating psychological impact on the patient. These risks may include changes in the individual's perception of self, stresses in relationships with friends or family, discrimination in the workplace or community, difficulties obtaining or keeping insurance, unfavourable adjustment of disability benefits, and other concerns related to privacy and confidentiality. A positive result could be beneficial, because sometime is preferable to know that you have a certain type of mutation in opposition with the anxiety generated by the absence of a clear answer. Also, the result of genetic testing could profoundly influence patient decision making with respect to finances (i.e., providing for inevitable hospice care), family planning (i.e., marriage or reproduction), and fostering personal relationships. Finally, the weighing of risks and benefits of genetic tests is a decidedly personal endeavour (36).

Although, the guidelines for presymptomatic genetic testing in ND vary among countries, some key elements include the following steps: neurologic neuro-psychologic and psychiatric evaluation, genetic counselling, sign informed consent document, psychological assessment, review of the potential impact of the test, disclosure of results in person and arrange post-result follow-up. For the testing will exclude patients with significant psychiatric disorders or those undergoing stressful life circumstances causing emotional upheaval. During the pre-test counselling

should be provided the explanations concerning implications of positive and negative results. All DNA determinations must be carried out independently at least twice and the results will be analysed after a genetic linkage computer analysis of haplotypes (the data from relatives must be analyzed anonymously to prevent the transmission of inappropriate information). Usually, the process is long and need many counselling sessions as the patient needs a psychological support. If the patient agrees the idea to inform other members of family about its genetic status, the patient could be accompanied by a relative during a minimum one session and also for the disclosure session when the results will be communicated. The diagnostic information must be given in a face- to-face session, because patient needs to discuss what this information may mean, even if the outcome is genetically uninformative. Long-term follow-up is essential, particularly for those who test positive for the gene. The testing are applied only to persons aged 18 years or older, must be totally voluntary and the results remain totally confidential (9,30-36).

Conclusions

The neurodegenerative diseases represent a group of diseases with late onset, invariable evolution to neurological and cognitive degradation, have not yet any pathogenic therapy and have a genetic component more or less important. In some such conditions the predictive genetic testing before onset of symptoms is possible, but is not available for general population. Such testing may be useful where a family member has already been diagnosed to carry a known mutation.

The genetic testing must be preceded by a genetic counselling respectful of the reli-

gious and cultural perspectives and traditions of individuals. Genetic counselling is complicated by a multitude of aspects, such as the apparition of a de novo mutation in a negative history family or familial factors, such early parental deaths, adoption, and illegitimacy.

Although the medical advances will be inherent and hopefully beneficial to the persons suffering from ND, the psychological and social support by sensitive, timely, and accurate counselling will remain an essential aspect in presymptomatic genetic testing.

REFERENCES

1. Magrone T, Marzulli G, Jirillo E, Immunopathogenesis of neurodegenerative diseases: current therapeutic models of neuroprotection with special reference to natural products, *Curr Pharm Des*, 2012; 18(1):34-42
2. Lopez OL, McDade E, Riverol M, Becker JT, Evolution of the diagnostic criteria for degenerative and cognitive disorders, *Curr Opin Neurol*. 2011; 24(6):532-541
3. Kearney JA, Genetic modifiers of neurological disease, *Curr Opin Genet Dev*, 2011, 21(3):349-353.
4. Kent A, Consent and confidentiality in genetics: whose information is it anyway?, *J Med Ethics*, 2003, 29 (1): 16-18
5. Wu-Chou YH, Chen YT, Yeh TH et al., Genetic variants of *SNCA* and *LRRK2* genes are associated with sporadic PD susceptibility: A replication study in a Taiwanese cohort, *Parkinsonism Relat Disord*, 2013, 19 (2): 251-255
6. Bagyinszky E, Youn YC, An SS, Kim S, The genetics of Alzheimer's disease, *Clin Interv Aging*, 2014; 9:535-51
7. Allan D, Ethical boundaries in genetic testing, *CMAJ*, 1996; 154:241-244.
8. *** Guidelines for genetic testing of healthy children - addendum: A joint statement with the Canadian College of Medical Geneticists, *Paediatr Child Health*, 2008; 13(4):311-312.
9. *** Commission of the European Communities, Brussels, 22 July 2009 - Council recommendation on measures to combat neurodegenerative diseases, in particular Alzheimer's, through joint programming of research activities, <http://www.alzheimer-europe.org/Policy-in-Practice2/EU-Action-on-Dementia>
10. McKhann GM, Knopman DS, Chertkow H et al., The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease, *Alzheimer's Dementia*, 2011; 7(3):263-9.
11. Ridge PG, Mukherjee S, Crane PK, Kauwe JS; Alzheimer's Disease Genetics Consortium, Alzheimer's disease: analyzing the missing heritability, *PLoS One*. 2013, 8(11):e79771.
12. Davinelli S, Intrieri M, Russo C et al., The Alzheimer's disease signature: potential perspectives for novel biomarkers; *Immun Ageing* 2011, 8:7 doi: 10.1186/1742-4933-8-7
13. Guerreiro R, Hardy J, Genetics of Alzheimer's disease, *Neurotherapeutics*, 2014;11(4):732-737.
14. Jenner P, Morris HR, Robbins TW et al., Parkinson's disease--the debate on the clinical phenomenology, aetiology, pathology and pathogenesis, *J Parkinsons Dis*. 2013; 3(1):1-11
15. Ariga H, Takahashi-Niki K, Kato I, Maita H, Niki T, Iguchi-Ariga SM, Neuroprotective function of DJ-1 in Parkinson's disease, *Oxid Med Cell Longev*, 2013;2013:683920. doi: 10.1155/2013/683920
16. Crosiers D, Theuns J, Cras P, Van Broeckhoven C, Parkinson disease: insights in clinical, genetic and pathological features of monogenic disease subtypes, *J Chem Neuroanat* 2011, 42(2):131-41
17. Malpass K, Parkinson disease: Unravelling the genetic contributors and their functional roles in sporadic PD, *Nature Rev Neurol* 2013, 9 (4), 182

18. Labbadia J, Morimoto RI, Huntington's disease: underlying molecular mechanisms and emerging concepts, *Trends Biochem Sci.* 2013, 38 (8): 378-85
19. Covic M, Ștefănescu D, Sandovici I, *Genetică medicală*, Ediția II-a, Polirom, Iași, 2011
20. Zheng Z, Diamond MI, Huntington disease and the huntingtin protein, *Prog Mol Biol Transl Sci*, 2012; 107:189-214
21. Kromberg JGR, Wessels T-M, Ethical issues and and Huntington's disease, *S Afr Med J*, 2013; 103 (12 Suppl. 1): 1023-1026
22. Durr A, Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond, *Lancet Neurol*, 2010; 9: 885-894.
23. Ilg W, Bastian AJ, Boesch S et al., Consensus paper: management of degenerative cerebellar disorders, *Cerebellum*. 2014;13(2):248-268
24. Sequeiros J, Martindale J, Seneca S, EMQN, Best Practice Guidelines for molecular genetic testing of SCAs, *Eur J Hum Genet*, 2010; 18 (11):1173-6.
25. Menon RP, Nethisinghe S, Faggiano S et al., The role of interruptions in polyQ in the pathology of SCA1, *PLoS Genet.* 2013, 9(7):e1003648.
26. Budworth H, McMurray CT. A brief history of triplet repeat diseases, *Methods Mol Biol.* 2013; 1010:3-17
27. Mand C, Gillam L, Duncan RE, Delatycki MB, "It was the missing piece": adolescent experiences of predictive genetic testing for adult-onset conditions, *Genet Med*, 2013, 15(8): 643-649
28. Mand C, Gillam L, Delatycki MB, Duncan RE. Predictive genetic testing in minors for late-onset conditions: a chronological and analytical review of the ethical arguments, *J Med Ethics.* 2012 38 (9):519-24.
29. Fenwick J, Are guidelines for genetic testing of children necessary?, *Fam Cancer* 2010; 9:23-5.
30. Wedderburn S, Panegyres PK, Andrew S et al. Predictive gene testing for Huntington disease and other neurodegenerative disorders, *Intern Med J*, 2013 43(12):1272-9
31. Marzuillo C, De Vito C, D'Andrea E, Rosso A, Villari P, Predictive genetic testing for complex diseases: a public health perspective, *QJM* 2014, 107 (2):93-7
32. Borry P, Stultiens L, Nys H, Cassiman JJ, Dierickx K, Presymptomatic and predictive genetic testing in minors: a systematic review of guidelines and position papers, *Clinic Genet*, 2006, 70 (5): 374-381.
33. Georgiou-Karistianis N, Long JD, Lourens SG, Stout JC, Mills JA, Paulsen JS; Predict-Hd Investigators and Coordinators Of The Huntington Study Group (Hsg), Movement sequencing in Huntington disease, *World J Biol Psychiatry.* 2014;15(6):459-71
34. Nalavade R, Griesche N, Ryan DP, Hildebrand S, Krauss S, Mechanisms of RNA-induced toxicity in CAG repeat disorders, *Cell Death Dis*, 2013 Aug 1;4:e752. doi: 10.1038/cddis.2013.276
35. Zimprich A, Biskup S, Leitner P et al., Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology, *Neuron* 2004;44:601- 607.
36. Roberts JS, Christensen KD, Green RC, Using Alzheimer's disease as a model for genetic risk disclosure: implications for personal genomics, *Clin Genet.* 2011;80(5):407-414.

NONSELECTIVE BETA-BLOCKERS IN PATIENTS WITH CIRRHOSIS: "THE THERAPEUTIC WINDOW"

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NONSELECTIVE BETA-BLOCKERS IN PATIENTS WITH CIRRHOSIS: "THE THERAPEUTIC WINDOW" (Abstract): For over 30 years, nonselective beta-blockers (NSBB) have been successfully used for preventing variceal bleeding in patients with cirrhosis and portal hypertension. Nevertheless, recent studies suggest that NSBB may be effective only within a particular "therapeutic window" in patients with advanced liver disease. Outside of this window, in early stages of cirrhosis and in very advanced cirrhosis, NSBB may be ineffective and even potentially harmful. In this paper we review the beneficial effects and potential harms of beta-blocker therapy in cirrhosis and underline the most recent recommendations for their use in very advanced cases of liver disease. **Keywords:** LIVER CIRRHOSIS; BETA-BLOCKERS; THERAPEUTIC WINDOW.

Pathophysiological basis for using nonselective beta-blockers (NSBB) in patients with cirrhosis. The development of portal hypertension in cirrhosis is mainly due to:

a) An increase in resistance to portal blood flow, because of architectural modifications in the liver, and

b) An increase in portal territory blood flow, due to splanchnic arteriolar vasodilatation.

Therefore, NSBB goal in cirrhosis is to reduce portal pressure by two essential mechanisms:

a) β_1 -adrenergic blockade, which decreases cardiac output, leading to reduced portal blood flow, and

b) β_2 -adrenergic blockade, followed by an increase in arterial splanchnic re-

sistance.

How did NSBB occur in the treatment of cirrhotic patient. The study of Lebrech et al. (3) published in 1981 in New England Journal of Medicine was the key-point in establishing the role of NSBB in the secondary prevention of variceal hemorrhage.

In 1987, the study of Pascal et al.(4) underlines the role of NSBB in the primary prophylaxis of variceal bleeding.

In 1991, Poynard et al.(5) meta-analysis together with other studies establishes that NSBB represent the first line therapy both in primary and in secondary prophylaxis of variceal hemorrhage.

The most important studies supporting NSBB usage in the treatment of cirrhotic patient are summarized by Phillip S. Ge., in

Journal of Hepatology from 2014 (1).

The indications of NSBB in cirrhosis

1. *Primary prevention of variceal hemorrhage:*

a) In cirrhotic patients with medium or large esophageal varices, NSBB lower the risk of first variceal bleeding (from 30% to 14%, compared to placebo) (6).

b) Primary prevention is not recommended in cirrhotic patients without esophageal varices, whatever the value of hepatic venous pressure gradient (HVPG).

2. *Secondary prevention of variceal hemorrhage:* NSBB reduce the rate of variceal rebleeding from 60% to approximately 40%.

3. *Other benefits:* NSBB might prevent the development of spontaneous bacterial peritonitis (SBP), by reducing intestinal permeability and bacterial translocation.

I. Pre-primary prophylaxis (to prevent the development of esophageal varices)

There are no data to confirm the benefit of using NSBB to prevent the development of esophageal varices, the most efficient method being the treatment of liver disease.

Studies showed that in patients without esophageal varices, NSBB (like *timolol*) have no role in preventing the development of esophageal varices, and the side effects are more frequent (7).

In conclusion, NSBB are not recommended in this set of patients.

II. Primary prevention (the prevention of first episode of variceal bleeding)

An important meta-analysis of 11 trials including almost 1200 patients evaluated the role of NSBB (*propranolol* or *nadolol*) in the primary prophylaxis of variceal haemorrhage (8).

The results showed that in cirrhotic pa-

tients with medium or large esophageal varices NSBB lower the risk of first variceal bleeding (from 30% to 14%, compared to placebo) (6).

Other studies show that one bleeding episode is avoided for every ten patients treated with NSBB. In conclusion, NSBB reduce the rate of bleeding-related mortality compared to the control group (9).

Upon the size of esophageal varices, the recommendations are as follows:

According to Baveno VI Consensus, in cirrhotic patients with *medium* or *large* esophageal varices NSBB are recommended to prevent the first episode of variceal hemorrhage (6).

Still, the risk of bleeding depends not only upon the size of esophageal varices, but also upon the presence of the marks of bleeding ("red signs") on the varices and on the severity of liver disease.

In patients with compensated liver disease (Child-Pugh A) and small esophageal varices without red signs, the risk of bleeding is 6%, while the patients with Child-Pugh C cirrhosis, with large esophageal varices with red signs have a risk of bleeding of 76% (10).

The set of cirrhotic patients with *small* esophageal varices are classified in two groups depending on the other factors which influence the risk of bleeding:

1. In patients with small esophageal varices and *low risk* of bleeding, which means without red signs and in the absence of severe liver disease (CHILD A), there are insufficient data that the use of NSBB would prevent the progression of esophageal varices. These patients may use NSBB to prevent the esophageal haemorrhage just in some individualized cases. The patients with small esophageal varices without therapy with NSBB must have endoscopy every 2 years, to evaluate the progression of

esophageal varices (11-13).

2. In cirrhotic patients with small esophageal varices and *high risk* of bleeding, meaning the presence of red signs and/or the presence of severe liver disease (CHILD-PUGH B or C), the treatment with NSBB is recommended (6,13).

III. Secondary prophylaxis of variceal bleeding

Secondary prevention means the prevention of rebleeding from esophageal varices, being addressed to those cirrhotic patients who have already had an episode of variceal haemorrhage.

Studies showed that patients who survive an episode of variceal haemorrhage have a high risk of rebleeding and death. In this set, untreated patients have a rebleeding rate of 60% at 1-2 years, with a mortality of 20-50% (14).

Besides, the prevention of rebleeding from esophageal varices was the first confirmed indication for using NSBB in the treatment of cirrhosis (the study of *Lebrech et al.* from 1981) (3).

It was demonstrated in trials that NSBB reduce the rebleeding rate from 60% to almost 40% (3,5,8).

According to the last guidelines, the combination of NSBB and *band ligation* is considered the maximal therapy for preventing rebleeding from esophageal varices. This provides the lowest rates of rebleeding, without major differences between the survival rates (compared to the patients treated only with NSBB). The early administration of NSBB may reduce the risk of bleeding till endoscopic band ligation. (6,15).

Other benefits of NSBB in cirrhosis include the prevention of other complications, like bleeding from portal gastropathy, the occurrence of ascites and of SBP (3,17).

The control of the efficiency of NSBB in cirrhosis targets:

a) *A hemodynamic response to NSBB* means a decrease in the HVPg below 12 mmHg or of 20% of the baseline value;

b) *The adrenergic response to NSBB* is defined as a decrease in the heart rate (measured 12-24 h after drug administration) of 25% of the baseline value;

c) *The dose of NSBB is decided* according to: a decrease in the heart rate (HR) of 25% of the basal value; the value of HR at rest not below 55/min; pay attention to the occurrence of side effects (3).

The side effects of NSBB

a) *Cardiac*: the exacerbation or precipitation of heart failure, symptomatic bradycardia, high grade heart block. The acute withdrawal of beta-blocker therapy can lead to accelerated angina, myocardial infarction and sudden death!

b) *Pulmonary*: increased airways resistance in patients with bronchospasm

c) *Circulatory*: the exacerbation of peripheral artery disease (claudication, absent pulses, cyanosis and gangrene)

d) *Metabolic*: in patients with diabetes mellitus, risk of severe insulin-induced hypoglycemia

e) *Other*: depression, fatigue and sexual dysfunction.

Contraindications to NSBB in cirrhotic patients

These may be classified as follows:

1) *General contraindications*

a) *Absolute* – severe heart failure, advanced heart block;

b) *Relative* – moderate chronic heart failure, asthma, chronic obstructive pulmonary disease, peripheral arterial disease, Raynaud syndrome, insulin-treated diabetes mellitus.

2) *Cirrhosis-related contraindications*

- a) Portopulmonary hypertension;
- b) Refractory ascites.

Is the therapy with NSBB in cirrhosis safe? How did this question occur? The same authors whose studies established the use of NSBB in the treatment of cirrhosis raised this problem!

In 2010, the study of Serste et al. (17,18) published in *Hepatology* concluded that NSBB should be contraindicated in cirrhotic patients with refractory ascites, leading to a shortening of survival.

Other authors, Wong and Salerno (19) showed that, regardless the design of the study, the safety of the treatment with propranolol in cirrhotic patients with refractory ascites is a problem.

In 2011, another trial of Lebec et al. (3) postulated that NSBB are associated with the paracentesis-induced circulatory dysfunction (PICD), leading to a reduced survival rate in this group of patients.

"The therapeutic window" hypothesis. Recent studies of Krag et al. (20) suggest that the efficiency of NSBB manifests just during a window in the disease, named "therapeutic window", in those patients with advanced cirrhosis.

Outside of this window, the therapy with NSBB may be ineffective in early stages of cirrhosis and potentially harmful in advanced cirrhosis (1).

Defining the term of "the therapeutic window". In early stages of cirrhosis, NSBB have no beneficial effect on survival and the adverse events may be more frequent (7).

These stages are defined by the following parameters: the esophageal varices are small or absent; intact cardiac reserve; sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS)

have baseline activity; the risk of gut bacterial translocation is low, and the risk of mortality is also low (7).

In decompensated cirrhosis, NSBB improves the survival rate, lowering the rate of esophageal bleeding and of bacterial translocation.

This stage of cirrhosis has the following characteristics: medium or large esophageal varices; ascites; the cardiac reserve is intact, but declining; SNS and RAAS have increased but not maximal activity, and the risk of bacterial translocation and of mortality are increased (1).

The final (terminal) stages of cirrhosis are characterized by refractory ascites; impaired cardiac reserve, with baseline hypotension; SNS and RAAS are maximally stimulated and can no longer maintain adequate blood pressure (BP); high risk of gut bacterial translocation and of mortality (7).

In these stages, NSBB decrease the rate of survival as a result of the negative impact on cardiac reserve, lowering the perfusion of vital organs and precipitating the hepatorenal syndrome (HRS).

Why NSBB are not indicated in refractory ascites? The study of Serste et al. (18) from 2011 show that in cirrhotic patients with refractory ascites NSBB may cause a higher risk of paracentesis-induced circulatory dysfunction, thus reducing the rate of survival (2). NSBB (like *propranolol*) would prevent the adaptive hemodynamic response after large volume paracentesis, subsequently inducing PICD.

The factors which close the "therapeutic window" are:

- a) The occurrence of refractory ascites and of SBP;
- b) Systolic BP below 95 mmHg;
- c) The presence of symptoms of orthostatic hypotension;

- d) The occurrence of septic episodes;
- e) Increased levels of serum creatinine.

The presented data show that the presence of medium or large esophageal varices opens the window of opportunity, which will be closed by the occurrence of refractory ascites, decreased medium BP and/or HRS. Taking into consideration the duration relatively limited of the "therapeutic window", some authors agree that NSBB should not be recommended in patients with low medical compliance and/or poor medical follow-up (21,22).

The initiation and continuation of NSBB therapy is ideal in those patients with home monitoring of BP and HR and who will have a close follow-up meaning frequent medical visits (23).

Concerning the "therapeutic window" hypothesis, the results from studies and the existing evidences are still conflicting.

For example, the study of Galbois et al. (24) published in *Hepatology* contradicts some of the previous data, suggesting that NSBB are not associated with an inadequate hemodynamic response in patients with cirrhosis and severe sepsis.

For now, the recommendations of **Baveno VI Consensus (6)** are the following:

1) The safety of beta-blockers administration in cirrhotic patients with *refractory ascites* and/or *hepatorenal syndrome* was not demonstrated;

2) The patients with *refractory ascites*

must be intensively monitored; if systemic hypotension or azotemia occur, we must reduce the dosage of NSBB or even discontinue the BB therapy;

3) If NSBB therapy is discontinued, the patient will need esophageal band ligation for preventing variceal bleeding;

4) Until practical data from randomised trials arrive, NSBB must be discontinued or the dose must be reduced in patients with refractory ascites if: systolic BP is below 90 mmHg; serum creatinine is raising and/or hyponatremia below 130 mmol/l occurs;

5) The consequence of discontinuing the BB therapy on the prevention of esophageal bleeding is not known;

6) If variceal bleeding or SBP occur after discontinuing the BB therapy, we may restart the treatment if the above parameters became normal.

CONCLUSIONS

In conclusion, the therapy with NSBB requires special attention in two clinical settings:

a) Decompensated cirrhosis in terminal stage, and

b) Refractory ascites.

It remains unclear if, after closing the "therapeutic window" of NSBB in cirrhosis, this would be a definitive closure or not, referring to the possibility of restarting the beta-blocker medication after a period of time.

REFERENCES

1. Ge PS, Runyon BA. The changing role of beta-blocker therapy in patients with cirrhosis. *J. Hepatol* 2014; 60: 643-653.
2. Krag A, Bendtsen F, Henriksen JH, Moller S. Low cardiac output predicts development of hepatorenal syndrome and survival in patients with cirrhosis and ascites. *Gut* 2010; 59: 105-110.
3. Lebrech D, Poynard T, Hillon P, Benhamou JP. Propranolol for prevention of recurrent gastrointestinal bleeding in patients with cirrhosis: a controlled study. *N Engl J Med* 1981; 305: 1371-1374.

4. Pascal JP, Cales P. Propranolol in the prevention of first upper gastrointestinal tract hemorrhage in patients with cirrhosis of the liver and esophageal varices. *N Engl J Med* 1987; 317: 856–861.
5. Poynard T, Cales P, Pasta L, Ideo G, Pascal JP, Pagliaro L, et al. Beta-adrenergic- antagonist drugs in the prevention of gastrointestinal bleeding in patients with cirrhosis and esophageal varices. An analysis of data and prognostic factors in 589 patients from four randomized clinical trials. Franco-Italian Multicenter Study Group. *N Engl J Med* 1991; 324: 1532–1538.
6. de Franchis R Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol.* 2015; 63(3): 743-752.
7. Groszmann RJ, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, et al. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *N Engl J Med* 2005; 353: 2254–2261.
8. D'Amico G, Pagliaro L, Bosch J. Pharmacological treatment of portal hypertension: an evidence-based approach. *Sem Liv Dis.* 1999; 19: 475–505.
9. La Mura V, Tosetti G, Primignani M, et al. Use of non-selective beta blockers in cirrhosis: the evidence we need before closing (or not) the window. *World J Gastroenterol.* 2015; 21(8): 2265-2268.
10. Krag A, Wiest R, Albillos A, Gluud LL. The window hypothesis: haemodynamic and non-haemodynamic effects of beta-blockers improve survival of patients with cirrhosis during a window in the disease. *Gut.* 2012; 61: 967–969.
11. Tsochatzis EA, Bosch J, Burroughs AK. New therapeutic paradigm for patients with cirrhosis. *Hepatology* 2012; 56: 1983–1992.
12. Merkel C, Marin R, Angeli P, Zanella P, Felder M, Bernardinello E, Cavallarin G, Bolognesi M, Donada C, Bellini B, Torboli P, Gatta A. A placebo-controlled clinical trial of nadolol in the prophylaxis of growth of small esophageal varices in cirrhosis. *Gastroenterol* 2004; 127: 476-484.
13. Garcia-Tsao G, Bosch J. Management of varices and variceal hemorrhage in cirrhosis. *N Engl J Med* 2010; 362: 823–832.
14. Mellinger JL, Volk ML. Multidisciplinary management of patients with cirrhosis: a need for care coordination. *Clin Gastroenterol Hepatol* 2013; 11: 217–223.
15. Senzolo M, Cholongitas E, Burra P, Leandro G, Thalheimer U, Patch D, Burroughs AK. beta-Blockers protect against spontaneous bacterial peritonitis in cirrhotic patients: a meta-analysis. *Liver Int* 2009; 29: 1189-1193.
16. Abraldes JG, Tandon P. The use of beta-blockers in advanced cirrhosis- where do we stand? *Curr Hepatology Rep* 2015; 14: 46-52.
17. Sersté T, Melot C, Francoz C, Durand F, Rautou PE, Valla D, Moreau R, Lebrech D. Deleterious effects of beta blockers on survival in patients with cirrhosis and refractory ascites. *Hepatol* 2010; 52: 1017-1022.
18. Serste T, Francoz C, Durand F, Rautou PE, Melot C, Valla D, et al. Betablockers cause paracentesis-induced circulatory dysfunction in patients with cirrhosis and refractory ascites: a cross-over study. *J Hepatol* 2011; 55: 794–799.
19. Wong F, Salerno F. Beta-blockers in cirrhosis: friend and foe? *Hepatol* 2010; 52: 811–813.
20. Krag A, Bendtsen F, Henriksen JH, Moller S. Low cardiac output predicts development of hepatorenal syndrome and survival in patients with cirrhosis and ascites. *Gut* 2010; 59: 105–110.
21. Sharma C, Fletcher KE, Zhang D, Kun YF, et al. Continuity of outpatient and inpatient care by primary care physicians for hospitalized older adults. *JAMA* 2009; 301: 1671-1680.
22. Dănulescu RM, Stanciu C, Trifan A: Evaluation of prognostic factors in decompensated liver cirrhosis with ascites and spontaneous bacterial peritonitis. *Rev Med Chir* 2015; 119 (4): 1018-1024.
23. Morando F, Maresio G, Piano S, Fasolato S, et al. How to improve care in outpatients with cirrhosis and ascites: a new model of care coordination by consultant hepatologists. *J Hepatol* 2003; 59: 257-264.
24. Galbois A, Das V, Thabut D, Maury E, Ait-Oufella H, Housset C, et al. Beta-blockers have no effect on outcomes in patients with cirrhosis and severe infections. *Hepatol.* 2011; 53: 1412–1413.

NADPH OXIDASE: STRUCTURE AND ACTIVATION MECHANISMS (REVIEW). NOTE I

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NADPH OXIDASE: STRUCTURE AND ACTIVATION MECHANISMS (Review). NOTE I (Abstract) NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase), with its generically termed NOX isoforms, is the major source of ROS (reactive oxygen species) in biological systems. ROS are small oxygen-derived molecules with an important role in various biological processes (physiological or pathological). If under physiological conditions some processes are beneficial and necessary for life, under pathophysiological conditions they are noxious, harmful. NADPH oxidases are present in phagocytes and in a wide variety of nonphagocytic cells. The enzyme generates superoxide by transferring electrons from NADPH inside the cell across the membrane and coupling them to molecular oxygen to produce superoxide anion, a reactive free-radical. Structurally, NADPH oxidase is a multi-component enzyme which includes two integral membrane proteins, glycoprotein gp91^{phox} and adaptor protein p22^{phox}, which together form the heterodimeric flavocytochrome b558 that constitutes the core of the enzyme. During the resting state, the multidomain regulatory subunits p40^{phox}, p47^{phox}, p67^{phox} are located in the cytosol organized as a complex. The activation of phagocytic NADPH oxidase occurs through a complex series of protein interactions. **Keywords:** REACTIVE OXYGEN SPECIES, NADPH OXIDASES, NOX, STRUCTURE, LOCALIZATION, FUNCTIONS.

Oxidative stress (SO) is essentially the result of an imbalance between the endogenous production of free radicals derived from oxygen (ROS) or nitrogen (RNS), and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants (1, 2, 3). This molecular imbalance in ROS metabolism has been proven both at the cellular level and throughout the body. ROS have an opposing, context-dependent dual role.

Under normal circumstances, the production and removal of ROS, that occurs in distinct extracellular or (sub)cellular spaces, are strictly controlled by effective endogenous defense mechanisms that block their excessive production. Some reactive oxygen species are necessary for life (especially superoxide anion radical O²⁻, hydrogen peroxide H₂O₂) (4) having a pivotal role in mediating important functions in normal cellular physiology by their contri-

bution to regulating different physiological processes such as cellular defense, signaling mechanisms, steroid synthesis, activation of G protein-coupled receptors, regulation of transcription factors, gene expression (5).

Overproduction of ROS results in oxidative stress with various pathological consequences such as atherosclerosis, hypertension, vascular remodeling after angioplasty, myocardial infarction, asthma, stroke, etc. (6, 7, 8, 9).

Sources of ROS. Of the many sources of ROS we mention the redox enzymes NADPH oxidase, mitochondrial respiratory chain, xanthine oxidase, lipoxygenase, and cyclooxygenase, which are continuously interacting with each other. In biological systems, ROS have the superoxide radical anion $O_2^{\cdot-}$ as one of the most important representative. A typical enzyme belonging to this redox group that generates ROS is NADPH oxidase, with important biological functions (10). NADPH oxidases are present in the higher life forms (animals, plants), but also in fungi, but not in prokaryotes and most unicellular eukaryotes. The enzyme is expressed in professional phagocytic cells, multiple types of blood cells (such as neutrophils, monocytes, eosinophils, macrophages, mast cells) (11) involved in antibacterial, antifungal, antimicrobial defense by reactions with free radicals; the enzyme is also involved in innate immunity (12). Phagocytic NADPH oxidase becomes active upon stimulation and then generates superoxide anions. An alternative scenario is that phagocytic NADPH oxidase directs the ions inside and outside the vacuole formed by endocytosis producing changes in pH and ion flow (13). NADPH oxidase and NADPH oxidase-like

enzyme also described in non-phagocytic cells have been identified in almost every type of tissue, but functionally distinct from phagocytic oxidases (14). Non-phagocytic NADPH oxidases are constitutively active enzymes that produce superoxide radical anion intracellularly in a slow and sustained manner and act as a signaling molecule influencing the transcription factors, molecules involved in inflammation, cell growth etc (15).

The cellular compartments in which NADPH oxidases are expressed include: the nucleus, endoplasmic reticulum, endosomes, phagosomes, mitochondria, and extracellular space (16).

Structure of NADPH oxidase. In mammals, NADPH oxidase is a plasma membrane-bound multicomponent complex (heteroprotein) that faces the extracellular space, consisting of 7 isoforms (homologues). ROS is produced by NADPH oxidase through NOX protein, thus differentiating the enzyme from other oxidases. NADPH oxidases are collectively referred to as the NOX family: NOX 1, NOX 2 (identical to membrane glycoprotein gp91^{phox}, also known as a subunit β , suffix phox refers to **phagocyte oxidase**) (17), NOX 3, NOX 4, NOX5 and associated dual oxidases – Duox 1 and Duox 2 (18, 19, 20). The catalytic subunits of NOX 1, NOX 2, NOX3 and NOX 4 form a macromolecular complex with p22^{phox} (known as subunit α) as a common enzyme activator subunit. NOX 5 Duox1 and Duox2 are p22^{phox}-independent isoforms. Duox 1 and 2 have additional peroxidase domains termed this way due to homology to peroxidases which catalyze dismutation of superoxide anion to hydrogen peroxide – H_2O_2 (13). Therefore, each of these isoforms has a core catalytic

subunit, the so-called NADPH-oxidase (NOX), two dual oxidase subunits (Duox) and 5 regulatory subunits.

Isoenzymes of NADPH-oxidase (NOX) are distinguished by the following features: subcellular localization, tissue and subcellular patterns of expression, structure (the nature of the catalytic subunits determines the adaptation of regulatory subunits), mechanism of enzyme activation, ROS-generating catalytic mechanism and functions (1, 10, 21).

Physiological functions of NADPH oxidases: the enzymes catalyze the transfer of electrons in cytosol across the membrane from NADPH, which oxidizes, to molecular oxygen, which is reduced to produce the superoxide radical anion ($O_2^{\cdot-}$), generator of other ROS species. The NADPH oxidases was originally identified as a component of innate host defence. In phagocytes, this complex enzyme is activated to produce superoxid anion and other secondarily derived ROS, wich promote killing of invading micro-organisms. Another non phagocytic NADPH oxidases system is present in almost every body organ being physiologically involved in signal transduction, cell proliferation and differentiation, posttranslational processing of proteins (4, 18).

Mechanism of action of NADPH oxidase Although all NOX enzymes have the same function in ROS generation, the activation and control mechanisms, the nature of the ROS produced, subunit requirements, and intracellular distribution varies between isoforms (22). Some NOX are membrane proteins with catalytic functions, other have cytosolic localization and regulatory functions (23). All NOX family members share a common core structure

made up of 6 transmembrane domains. The catalytic core of the classic phagocytic enzyme – NADPH-oxidase (NOX 2) as of the other isoforms consists of two membrane-integrated proteins (subunits): gp91^{phox}, glycosylated protein (24), (considered the patriarch of NOX family) and p22^{phox} (non-glycosylated adapter protein) which together form a large subunit, the flavocytochrome b558 complex (heterodimeric flavohemoprotein b558). Gp91^{phox} subunit consists of 6 transmembrane domains and the cytoplasmic C-terminal region which contains two binding sites for FAD (flavin adenine dinucleotide) and for the electron donor NADPH. All NADPH oxidases except NOX 5, Duox 1 and Duox 2 have similar topological structure of the catalytic core gp91^{phox}. The N-terminal domain of NOX 5 binds calcium, Duox1 and Duox 2, in addition to the NOX 5 structure present at the N-terminal transmembrane α -helix a domain homologous to peroxidase (25).

The regulatory subunits play important roles in: maturation and expression of NOX and Duox subunits in biological membranes, and in enzyme activation and spatial organization of the various components of the enzyme complex (26). Some isoforms of NADPH oxidase contain GTP-ase (guanine triphosphate hydrolase), Rac 1 and Rac 2, which modulate their activity (27).

The activation of phagocyte NADPH oxidase occurs through a series of complex protein interactions in response to numerous agonists (27). In unstimulated cells, components p40^{phox}, p47^{phox} and p67^{phox} are grouped in the cytosol into a complex (25). When the resting cell is exposed to a variety heterogeneous stimuli (chemical, physical, environmental, biological factors)

(24), the peripheral cytosolic component becomes highly phosphorylated able to form a complex which migrates to the membrane where it associates with flavocytochrome b558 and together with other components – p40^{phox}, p47^{phox} and p67^{phox} – forming the active oxidase which transfers electrons from the substrate (NADPH) to molecular oxygen with formation of superoxide radical anion (27, 28), precursor of other toxic ROS (17). The activation process is strictly controlled, involving in addition to phosphorylation multiple conformational changes. This enzymatic complex is involved in many key biological functions such as cell growth, proliferation, differentiation, migration, defense, signaling and regulation of gene expression. Excessive ROS production attributed to the increased NOX activity, causing cellular oxidative stress, may contribute to the oc-

currence of a wide variety of diseases: vascular disease, inflammation, endothelial dysfunction, cell proliferation, fibrosis, angiogenesis, cardiovascular remodeling, cardiac aging, renal remodeling in hypertension, atherosclerosis, diabetes, heart failure, myocardial ischemia-reperfusion injury.

CONCLUSIONS

NADPH-oxidases is a proteic complex that, being stimulated, is capable to produce O²⁻, by transferring an electron from NADPH to O₂. It is a multicomponent family enzyme, with a complex structure made of membrane proteins complex, with catalytic properties; in the same time, the enzyme has cytosolic structures with regulatory properties. The tissue distribution of NADPH-oxidases and their activating mechanisms are of large variety.

REFERENCES

1. Schram A, Matusik P, Osmenda G, Guzik TJ. Targeting NADPH oxidases in vascular pharmacology. *Vascul Pharmacol* 2012; 56(5-6): 216-231.
2. Crețu E, Trifan A, Aprutosaie AC, Miron A: 15-lipoxygenase inhibition superoxide and hydroxyl radicals scavenging activities of Cedrus Brevifolia bark extract. *Rev Med Chir Soc Med Nat*, 2013; 117(1): 250-6.
3. Ciocoiu M, Miron A, Bădescu M: New phenolic extracts for oxidative stress treatment in experimental diabetes. *Rev Med Chir Soc Med Nat*, 2008; 112(3): 757-63.
4. Amanso AM, Griendling KK. Differential roles of NADPH oxidase in vascular physiology and pathophysiology. *Front Biosci (Schol Ed)* 2012; S4: 1044-1064.
5. Sedeek M, Nasrallah R, Touyz RM, Hébert RL. NADPH oxidases, reactive oxygen species, and the kidney: Friend and foe. *JASN* 2013; 14(10): 1512-1518.
6. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species and hypertension. Clinical implications and therapeutic possibilities. *Diabetes Care* 2008; 31 (supplement 2): S170 – S180.
7. Konior A, Schram A, Czesnikiewicz-Guzik M, Guzik TJ. NADPH oxidases in vascular pathology. *Antioxid Redox Signal* 2014; 20(17): 2794-2814.
8. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malic AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 2014; 20(7): 1126-1167.
9. Maiellaro-Rafferty K, Weiss D, Joseph G, Wan W, Gleason RL, Taylor WR. Catalase over expression in aortic smooth muscle presents pathological mechanical changes underlying abdominal aortic aneurysm formation. *Am J Physiol Heart Circ Physiol*, 2011; 301(2): H355-H362.

10. Nauseef WM. Biological roles for the nox family NAD(P)H oxidases. *J Biol Chem* 2008; 283(25): 16961 – 16965.
11. Bernard K, Hecker L, Luckhard TR, et al. NADPH Oxidases in Lung Health and Disease. *Antioxid Redox Signal*, 2014; 20(17): 2838-2853.
12. Smirnov A, Daily KP, Criss AK. Assembly of NADPH Oxidases in Human Neutrophils is Modulated by the Opacity-Associated Protein Expression State of *Neisseria gonorrhoeae*. *Infect. Immunol.*, 2014, Vol. 82, No. 3: 1036-1044.
13. Segal AW. The function of the NADPH oxidase of phagocytes and its relationship to other NOX inplants, invertebrates, and mammals. *Int J Biochem Cell Biol* 2008; 4(4-3): 604-618.
14. Zhang L, Wu J, Duan X, et al. NADPH Oxidase: A potential Target for Treatment of Stroke. *Oxidative Medicine and Cellular Longevity*. 2016, <http://dx.doi.org/10.1155/2016/5026989>.
15. Cosentino F, Francia P, Camici GG et al. Final common molecular pathways of aging and cardiovascular disease: role of the p66Shc protein. *ATVB* 2008; 28(4): 622-628.
16. Lassègue A, Griendling KK. NADPH oxidases: functions and pathologies in vasculature. *ATVB* 2010; 30(4): 653-662.
17. Segal BH, Veys P, Malech H, et al. Chronic Granulomatous Disease: Lessons from a Rare Disorder. *Biol Blood Marrow Transplant* 2011, Vol. 17, Issue 1, supplement, S123-S131.
18. Lassègue B, San Martin A, Griendling KK. Biochemistry, Physiology, and Pathophysiology of NADPH Oxidases in the Cardiovascular System. *Circ Res* 2012; 110: 1364-1390.
19. Lassègue B, Martin AS, Griendling KK. Biochemistry, physiology, and pathophysiology of NADPH oxidases in cardiovascular system. *Circ Res* 2012; 110(10): 1364-1390.
20. Sumimoto H. Structure, regulation and evolution of NOX-family NADPH oxidases that produce reactive oxygen species. *FASEB J* 2008; 27(13): 3249-77.
21. Takac I, Schroder K, Brandes RP. The nox family of NADPH oxidases: friend or foe of the vascular system? *Curr Hypertens Rep* 2012; 14(1): 70-78.
22. Rodiño-Janciro GK, Paradelo-Dobarro B, Castiñeiras-Landeira MI, Raposeiras-Roubin S, González-Juanatey JR, Alvarez E. Current status of NADPH oxidase research in cardiovascular pharmacology. *Vasc Health Ris Manag* 2013; (9): 401-428.
23. Panday A, Sahoo MK, Osorio D, et al. NADPH oxidases: an overview from structure to innate immunity-associated pathologies. *Cell Mol Immunol* 2015; 12: 5-23.
24. Garrido AM, Griendling KK. NADPH oxidases and Angiotensin II Receptor Signaling. *Mol Cell Endocrinol* 2009; 302(2): 148-158.
25. Pendyala S, Usatyuc PV, Gorshkova IA, Garcia JG, Natarajan V. Regulation of NADPH oxidase in vascular endothelium: the role of phospholipase, protein kinase, and cytoskeletal proteins. *Antioxid Redox Signal* 2009; 11(4): 841-860.
26. Purushothaman D, Sarin A. Citokine-dependent regulation of NADPH oxidase activity and the consequence for activated T cell homeostasis. *J Exp Med* 2009; 206(7): 1515-1523.
27. Kim Y-W, Byzova TV. Oxidative stress in angiogenesis and vascular disease. *Blood* 2013; 123(5): 625-631.
28. Pandey A, Sahoo MK, Osorio D, Batra S. NADPH oxidase: an overview from structure to innate immunity-associated pathologies. *Cell Mol Immunol* 2015; 12: 5-23.

ANEMIA IN INFLAMMATORY BOWEL DISEASE MORE THAN AN EXTRAINTESTINAL COMPLICATION

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ANEMIA IN INFLAMMATORY BOWEL DISEASE: MORE THAN AN EXTRAINTESTINAL COMPLICATION (Abstract). The most common hematologic complication of inflammatory bowel disease (IBD) - ulcerative colitis and Crohn's Disease is anemia. Anemia in patients with IBD may be a result of iron, vitamin B12 or folate deficiency; anemia of chronic disease and hemolytic anemia are other causes in these patients. Factors contributing to the development of anemia include chronic gastrointestinal blood loss, vitamin B12 malabsorption secondary to terminal ileitis, folate deficiency as a result of sulfasalazine therapy. Approximately 30% of patients with IBD have hemoglobin levels below 12g/dl. The risk of developing anemia relates to disease activity, given that blood loss and inflammatory anemia are triggered by intestinal inflammation. In the management strategy of IBD patients with anemia it is important to distinguish between the different types of anemia in order to decide an appropriate manner of treatment. **Keywords:** INFLAMMATORY BOWEL DISEASE, ANEMIA, IRON DEFICIENCY, INFLAMMATORY ANEMIA.

Crohn's disease and ulcerative colitis are chronic inflammatory diseases of the gastrointestinal tract, mainly affecting the small intestine and colon, which progress with relapse and remission (1). IBD is associated with extraintestinal manifestation in more than 25% of cases, including malnutrition and anemia (1, 2).

Anemia is a clinically significant state which could impair quality of life or work capability and it represents a serious comorbid condition associated with other diseases or even death (1, 3). There are two major types of anemia in IBD patients: iron defi-

ciency anemia and inflammatory anemia called until recently anemia of chronic disease. Moreover, mixtures of the two types are commonly found in these patients (3, 4, 5).

Epidemiology of anemia in IBD. The reported prevalence of anemia in IBD is highly variable ranging from 15% up to 75% depending both on patient subgroup and definition of the disease. In hospitalized patients to prevalence is up to 70%, while in outpatients the reported prevalence is only 15% (4, 6).

Clinical manifestation. Patients may present with a diversity of symptoms and

Anemia in inflammatory bowel disease more than an extraintestinal complication

sign among which the most relevant are weakness, fatigue, dyspnea, tachycardia, headaches. These signs overlap the signs of the underlying disease (7, 8, 9) (tab. I).

Causes of anemia in IBD. The main types of anemia in IBD patients are iron deficiency anemia and inflammatory anemia or anemia of chronic disease (3).

TABLE I
Clinical consequences of anemia in IBD patients

Fatigue	Motility disorder	Impairments in quality of life
Dyspnea	Nausea	Loss of libido / impotence
Tachycardia	Anorexia	Impairments in physical condition
Headache	Menorrhagia/amenorrhea	Reduce energy efficiency during physical activity
Dizziness	Weight loss	Impairments in cognitive function

Iron deficiency anemia mainly results from chronic intestinal blood loss due to chronic inflammation of the intestinal mucosa and from reduced iron uptake from enterocytes (impaired absorption) (6, 7). Intestinal inflammation leads to a decreased serum iron level and therefore to a reduced availability of iron for erythropoiesis (6, 9). In addition, inflammatory status causes decreased iron uptake from the intestinal mucosa, hence, providing a multi-part two-way pathophysiologic mechanism between iron deficiency and inflammation (3, 4).

In patients with ileal Crohn's disease, absorption of vitamin B12 and/or folate may be impaired and all these conditions frequently overlap (6, 10). Similarly, anemia in IBD patients may be induced by

specific therapy-thiopurines, 5-ASA derivatives and/or by hemolysis (6, 9, 11) (tab. II).

Anemia workup. Full blood count, serum ferritin and C-reactive protein (CRP), are minimum requisite to identify anemia, an inflammatory relapse or iron deficiency at an early stage (7). Diagnostic measurement of blood count and CRP has been part of screening recommendations in IBD patients (11, 12). Patients in remission should be followed-up every 6 to 12 months, while active disease needs measurements at least every 3 months (11). Patients with extensive ileal Crohn's disease, ileal resection or ileal-anal pouch need proper follow-up. Serum vitamin B12 and folic acid levels should be measured every 6 to 12 months (7, 8, 9) (tab. III).

TABLE II
Etiology of anemia in IBD patients

Iron deficiency	Regular
Anemia of chronic disease	
Vitamin B ₁₂ deficiency	Common
Folate deficiency	
Drug induced 5-ASA; Thiopurine	
Hemolysis	Infrequently
Myelodysplastic syndrome	
Aplastic anemia	

The initial workup of anemia starts with the evaluation of MCV in order to distinguish between microcytosis (iron restricted anemia - iron deficiency and/or inflammatory anemia) and macrocytosis (B12 or folate deficiency) and also normocytosis (anemia of chronic disease) (8).

Platelet and white blood cell counts should be measured to reveal pancytopenia (12).

Serum ferritin level reflects the iron stores. Iron deficiency anemia is present when serum ferritin level is below 30 µg/L and hemoglobin level below 12g/L for non-pregnant females and below 13g/L for males (8).

Therefore, serum ferritin level can be used to detect iron deficiency; a drawback is that ferritin is a marker of acute inflammation and has to be evaluated together with CRP, as elevated ferritin may be due to a flair of inflammation or a superimposed infection (8, 12).

TABLE III
Serum markers of anemia
in IBD patients

Mean corpuscular volume (MCV)
CRP
Serum Vitamin B₁₂
Serum Folate
Ferritin
Transferrin
Transferrin saturation (T-Sat)
Hepcidin
Soluble transferrin receptor concentration (StfR)

The diagnosis of iron deficiency anemia in IBD patients may be difficult mainly when both iron and inflammatory anemia are present. In this case, laboratory measures of iron status may be unreliable, as the presence of intestinal inflammation affects the parameters of iron metabolism (12).

In IBD patients with anemia even transferrin concentration may not be elevated as in pure iron deficiency anemia, due to the low albumin levels found in patients with intestinal inflammation (13).

Active IBD determines elevated hepcidin levels. Hepcidin blocks intestinal iron absorption and also reduces the release of iron from stores resulting in inflammatory anemia (11, 14).

Thus, a serum ferritin level between 30 and 100 µg/L in the presence of anemia and high CRP level indicates a mixture of iron deficiency and inflammatory anemia (14). On the contrary, a serum ferritin level over 100 µg/L together with anemia and elevated CRP level indicates pure inflammatory anemia (14).

T-Sat is a measure of the iron content in the circulating transferrin. A T-Sat below 16% indicates a suboptimal source of iron for erythropoiesis (14). T-Sat level provides an indirect indication of the degree of iron use in the bone marrow but does not provide evidence of the iron stores (2). In IBD, T-Sat is used in the anemia workup in combination with ferritin and CRP, at a lower cut-off point <16% (along with ferritin level <30 µg/L suggests iron deficiency) (13).

Testing for increased sTfR distinguishes between these two types of anemia but it is not generally available yet. A high diagnostic power of sTfR or $\frac{sTfR}{\log \text{ ferritin}}$ ratio in differentiating iron deficiency from inflammatory anemia has been suggested by several studies (13).

Drugs commonly used in IBD treatment may have myelosuppressive effects: direct (thiopurine) or indirect (antifolic effect of sulfasalazine) (11). Sulfasalazine inhibits erythropoiesis by different mechanisms: hemolysis, aplasia and/or folate absorption (12, 13). A mild anemia may be detected in patients treated with thiopurine drugs (aza-

thioprine or mercaptopurine) (8, 14).

Management of anemia in IBD. The correlation between disease activity and level of anemia is well known. Consequently, the most significant measure in the treatment of patients with IBD and anemia is the specific treatment of the underlying disease (12, 15).

Inflammatory anemia is a common cause of anemia among hospitalized IBD patients with active disease. Treatment of non-iron deficiency anemia may include correction of IBD specific treatment, nutritional supplements, treatment of infections and, in selected cases, erythropoiesis-stimulating agents (13, 14).

In IBD patients on biological treatment (infliximab, adalimumab) the response to therapy has been shown to determine improvement of erythropoiesis, by significantly increasing the serum transferrin level (2). Colectomy is reserved for intrac-

table, anti-TNF α -resistant ulcerative colitis. Folic acid and vitamin B12 deficiencies, another cause of non-iron deficiency anemia, should be treated in order to avoid anemia especially in patients with Crohn's disease, ileal resection and ileal-anal pouch (14).

Red blood cell transfusion may be considered when hemoglobin is < 7g/dL or above if risk factors and/or fatal comorbidities are present (9, 15). Nowadays transfusions in IBD patients are restricted to the severe attack of ulcerative colitis with severe anemia and hemodynamic instability, surgery for Crohn's disease, in the presence of severe comorbidities (14, 15).

Iron replacement treatment should be started immediately after iron deficiency anemia is diagnosed. Treatment options for iron deficiency anemia are intravenous iron supplementation or oral iron administration (2, 16) (fig 1).

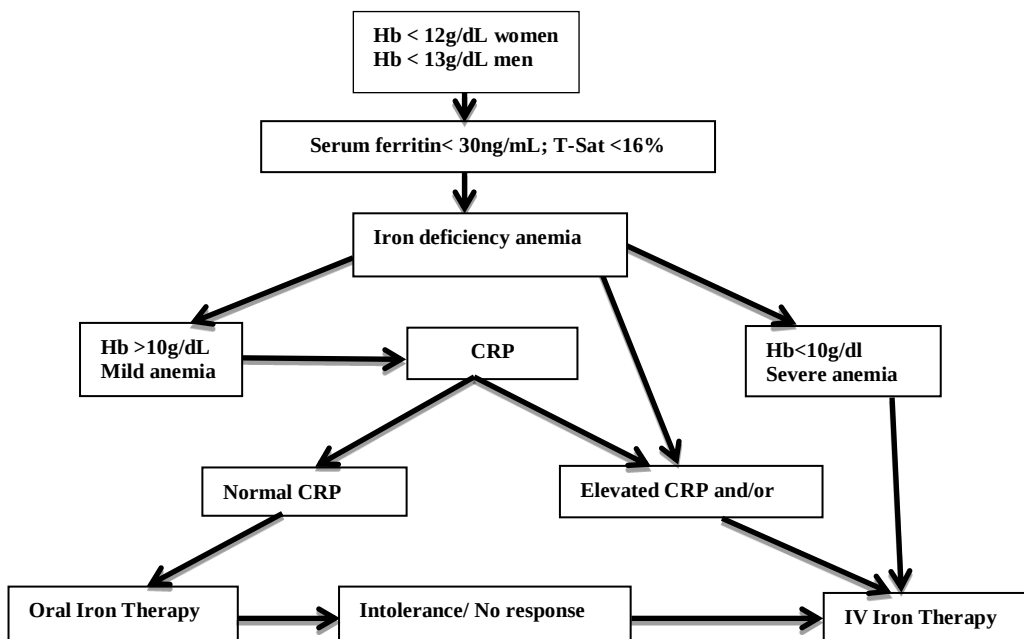


Fig. 1. Treatment strategies for IBD patients with iron deficiency anemia.

The therapeutic goals in iron deficiency anemia are to normalize hemoglobin, ferritin, and T-Sat levels, replenish iron stores (ferritin > 100 µg/L) and improve quality of life (17). After the initiation of martial therapy, reticulocyte count will rise in the next 7-9 days and hemoglobin in one month (18).

Oral iron therapy is indicated in patients with mild anemia, hemoglobin > 10g/dL and remission in which oral iron absorption is not affected (17). There are many limitations of oral iron therapy: intolerance, non-compliance, worsens disease activity (14, 19).

Therefore, intravenous iron is the treatment of choice in iron deficiency anemia. According to the guidelines and consensus statement, in IBD patients intravenous iron supplementation is the preferred route. The advantage of intravenous iron therapy is better tolerance, compliance (usually single dose) and rapid reversal of anemia (18, 20, 21).

Ganzoni formula is useful to estimate iron requirements (2): Iron deficit (mg) = Body weight (kg) x [target Hb - actual (g/dL) x 2.4 + iron stores (500mg)

CONCLUSIONS

Our analysis of the literature studies revealed that anemia is the most common extraintestinal complication of IBD. It can increase the hospitalization rate in IBD patients and can affect quality of life and work capability.

An important goal for the IBD practitioner is to use anemia markers in order to accomplish the highest diagnosis yield of anemia in IBD patients. Anemia in IBD is usually a complex manifestation of a mixed type of iron deficiency and inflammatory conditions. The gastroenterologist should keep in mind other causes of anemia especially in small intestine involvement- vitamin B12 and folate deficiency. The pathophysiological causes of anemia in IBD include malabsorption, malnutrition, inflammation, intestinal resection, drug adverse events.

Iron deficiency anemia is common in IBD patients and is difficult to diagnose in the presence of inflammation. Intravenous iron therapy is a preferable option especially in active IBD patients and moderate-severe iron deficiency anemia.

REFERENCES

1. Gomollón F, Gisbert JP. Anaemia and inflammatory bowel diseases. *World J Gastroenterol* 2009; 15: 4659-4665.
2. Sindhu K, Muhammad B, Tauseef A. Iron deficiency anemia in inflammatory bowel disease. *World J Gastrointest Pathophysiol* 2015; 6(3): 62-72.
3. Zhu A, Kaneshiro M, Kaunitz JD. Evaluation and treatment of iron deficiency anemia: a gastroenterological perspective. *Dig Dis Sci* 2010; 55: 548-559.
4. Danila G, Alfredo JL. Anemia in inflammatory bowel disease: A neglected issue with relevant effects. *World J Gastroenterol* 2014; 20(13): 3542-3555.
5. Dumitrescu G, Dranga M, Pintilie IA, Nedelciuc O, Mihai C, Prelipcean CC. The prevalence of anaemia in patients with inflammatory bowel diseases in North-Eastern Romania. *Rev Med Chir Soc Med Nat Iasi* 2012; 116: 968-974.

Anemia in inflammatory bowel disease more than an extraintestinal complication

6. Filmann N, Rey J, Schneeweiss S et al. Prevalence of anemia in inflammatory bowel diseases in European countries: a systematic review and individual patient data meta-analysis. *Inflamm Bowel Dis* 2014; 20: 936-945.
7. Pantelis O, Ioannis EKA. Diagnosing anemia in inflammatory bowel disease: Beyond the established markers. *J Crohn's and Colitis* 2011; 5: 381–391.
8. Wells CW, Lewis S, Barton JR, Corbett S. Effects of changes in hemoglobin level on quality of life and cognitive function in inflammatory bowel disease patients. *Inflamm Bowel Dis* 2006; 12: 123-130.
9. Gasche C, Lomer MCE, Cavill I, Weiss G. Iron, anaemia, and inflammatory bowel diseases. *Gut* 2004; 53:1190–1197.
10. Kulnigg S, Gasche C. Systematic review: managing anemia in Crohn's disease. *Aliment Pharmacol Ther* 2006; 24:1507–1523.
11. Palle B. Anemia in Inflammatory Bowel Diseases is much more than Levels of Hemoglobin. *J Gastrointest Liver Dis* 2015; 24(2): 145-146.
12. Jürgen S, Franz H, Axel U. Diagnosis and management of iron deficiency anemia in patients with IBD. *Nature Reviews Gastroenterol Hepatol* 2010; 7: 599-610.
13. Walker AM, Szeke P, Bianchi LA, Field LG, Sutherland LR, Dreyer NA. 5-Aminosalicylates, sulfasalazine, steroid use, and complications in patients with ulcerative colitis. *Am J Gastroenterol* 1997; 92: 816-820.
14. Zehra BP, Cem Ç, Mahmut A et al. An Evaluation of the Correlation between Hepcidin Serum Levels and Disease Activity in Inflammatory Bowel Disease. *Gastroenterol Res Pract* 2015, Article ID 810942, 4 pages, <http://dx.doi.org/10.1155/2015/810942>.
15. Bergamaschi G, Di Sabatino A, Albertini R et al. Prevalence and pathogenesis of anemia in inflammatory bowel disease. Influence of anti-tumor necrosis factor-alpha treatment. *Haematol* 2010; 95: 199–205.
16. Cucino C, Sonnenberg A. Cause of death in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2001; 7: 250-255.
17. Mei Z, Cogswell ME, Parvanta I, Lynch S, Beard JL, Stoltzfus RJ, Grummer-Strawn LM. Hemoglobin and ferritin are currently the most efficient indicators of population response to iron interventions: an analysis of nine randomized controlled trials. *J Nutr* 2005; 135: 1974-1980.
18. Oldenburg B, Koningsberger JC, Van Berge Henegouwen GP, Van Asbeck BS, Marx JJ. Iron and inflammatory bowel disease. *Aliment Pharmacol Ther* 2001; 15: 429-438.
19. Marineata A, Rezus E, Mihai C, Prelipcean CC. Extraintestinal manifestations and complications in inflammatory bowel disease. *Rev Med Chir Soc Med Nat Iasi* 2014; 118(2): 279-288.
20. Kulnigg S, Teischinger L, Dejaco C, Waldhör T, Gasche C. Rapid recurrence of IBD-associated anemia and iron deficiency after intravenous iron sucrose and erythropoietin treatment. *Am J Gastroenterol* 2009; 104: 1460-1467.
21. Axel UD, Christoph G, Dominik B, Gunnar B, Jürgen S, Stephan V. ECCO Guideline/Consensus Paper; European Consensus on the diagnosis and management of Iron deficiency and anemia in inflammatory bowel diseases. *J Crohn's and Colitis*, 2015, 1–12.

ATOPIC DERMATITIS - CLINICAL EPIDEMIOLOGY AND IMMUNOLOGICAL CORRELATIONS

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ATOPIC DERMATITIS - CLINICAL EPIDEMIOLOGY AND IMMUNOLOGICAL CORRELATIONS (Abstract) **Aim:** Medical records from 2010-2014 were retrospectively reviewed and analyzed in view of determining the correlations between the clinical severity of atopic dermatitis (AD) and total IgE level, eosinophilia, place of residence, family history of atopy, type of birth, and natural or artificial feeding. **Material and Methods:** Following medical record review, 296 cases diagnosed with AD were included in the study. Statistical analysis was performed using SPSS v20 for determining the frequency and testing the hypotheses, for $p < 0.05$, by t tests and One-Way ANOVA. **Results:** Of the 296 cases, 53% were male children and 47% female children aged 1 month to 16 years. According to total serum IgE level, 49.3% of patients had elevated IgE levels, 20.6% normal levels and in 30.03% of cases it was not determined. According to the SCORAD, children had mild AD in 20% of cases, moderate in 70%, and severe in 10%. The independent samples t tests showed a statistically significant difference between the means demonstrating correlations between IgE level and place of residence ($p < 0.01$), family history of atopy ($p < 0.01$), baby feeding ($p < 0.01$), and by one-way ANOVA for SCORAD ($p < 0.05$). **Conclusions:** Atopy in AD can be influenced by complex factors, both internal and environmental, but this remains a controversial topic. External factors acting on a background genetically predisposed to atopy trigger the manifestation of AD. **Keywords:** ATOPIC DERMATITIS, FOOD ALLERGIES, FAMILY AGGREGATION, ENVIRONMENTAL FACTORS.

Atopic dermatitis (AD) is a chronic inflammatory skin condition characterized by intense itching. In 2003, the European Academy of Allergology and Clinical Immunology revised nomenclature of allergic diseases and proposed the term atopic eczema for the Immunoglobulin E-mediated skin inflammatory disorder occurring in genetically predisposed individuals (1, 2).

Atopic eczema develops in early child-

hood. About one in five school-age children have atopic eczema. Quality of life is altered as it affects sleep and concentration, school attendance, and self-image. AD starts at ~ 2 months (90% before the age of 5 years). Prevalence is 10-25% in the US, Northwestern Europe, and Australia (3).

In the etiopathogenesis of AD are involved the following factors: genetic predisposition, family aggregation, low expo-

sure to infectious agents and/or commensal microbiota - hygiene hypothesis and rise in living standards - "Western lifestyle" (4).

The genetic risk factor is represented by loss-of-function mutations in the filaggrin gene (FLG), an important epidermal barrier protein and precursor of natural moisturizing factor, involved in epidermal integrity (3,4).

Seventy percent of the patients with AD have a family history of atopy. If both parents are atopic their children have an 81% risk for developing atopy; when one parent is atopic and the other parent is non-atopic the risk is of 56%; frequency of atopic manifestations in other family members (first-degree relatives) is associated with a 28% risk (5).

Among the factors that could favor the onset and progression of AD lesions, the most commonly incriminated are: dietary factors - cow milk, eggs, peanuts, nuts, soy, wheat, fish, seafood, airborne allergens, infectious agents - *Staphylococcus aureus*, *Pytirosporum ovale*, contact factors - cleaning agents, astringent solutions, perfumes, harsh soaps and detergents, tight and abrasive clothing and physical factors - extreme temperatures, emotional stress (6).

MATERIAL AND METHODS

This retrospective study analyzed the data collected from medical records from 2010 - 2014. There were 296 cases of AD of which 53.05% were male and 46.95% female children from all Moldavian regions. Distribution of cases by county of residence was the following: 73.99% Iasi county, 8.44% Neamt county, 4.72% Botosani and Vaslui counties, 4.05% Suceava county, and 0.67% Vrancea and Galati counties.

The diagnosis of AD was made according to Haniffin and Rajka criteria, and the severity of lesions was based on SCORAD

score calculation according to the international protocol and classified as mild (<15), moderate (15-40) and severe form > 40 (7).

Inclusion criteria were patients at first presentation to our clinic with or without a personal history of AD. The following data were analyzed: gender, place of residence (rural or urban), family history, personal history (type of delivery and feeding), previous medical history (recurrent allergic reactions and respiratory infections), SCORAD, total IgE and specific IgE levels.

Statistical analysis was performed using SPSS v20 for determining the frequency and testing the hypotheses, for $p < 0.05$.

RESULTS

Of the 296 AD cases 37.5% were recorded in 2014, 19.26% in 2010, 17.22% in 2013, 13.18% in 2011 and 12.84% in 2012 (fig. 1). Fifty-three percent of the children were male and 47% female, aged from 1 month to 16 years. An incidence of 30.3% in the first 6 months of life and of 80.8% in the first 3 years was found (fig. 2), values close to those reported in the literature (7).

These patients were diagnosed according to Haniffin and Rajka criteria corroborated with the clinical and laboratory findings at the first admission (8). Thus, total serum IgE level was elevated in 49.32% of patients, normal in 20.61% of patients, and in 30.07% it had not been determined. Studies report a higher frequency of AD in urban than in rural areas (9, 10, 11). In our study, 52% were urban patients and 48% rural patients, average of 1.52, SD = 0.50 (standard deviation) and variance of 0.250. The independent samples t-test showed that for $t(294) = -0.98$, the p value was greater than 0.01 but F test rejected the null hypothesis, therefore the place of residence influences AD (tab. I).



Fig. 1 Distribution of cases by year

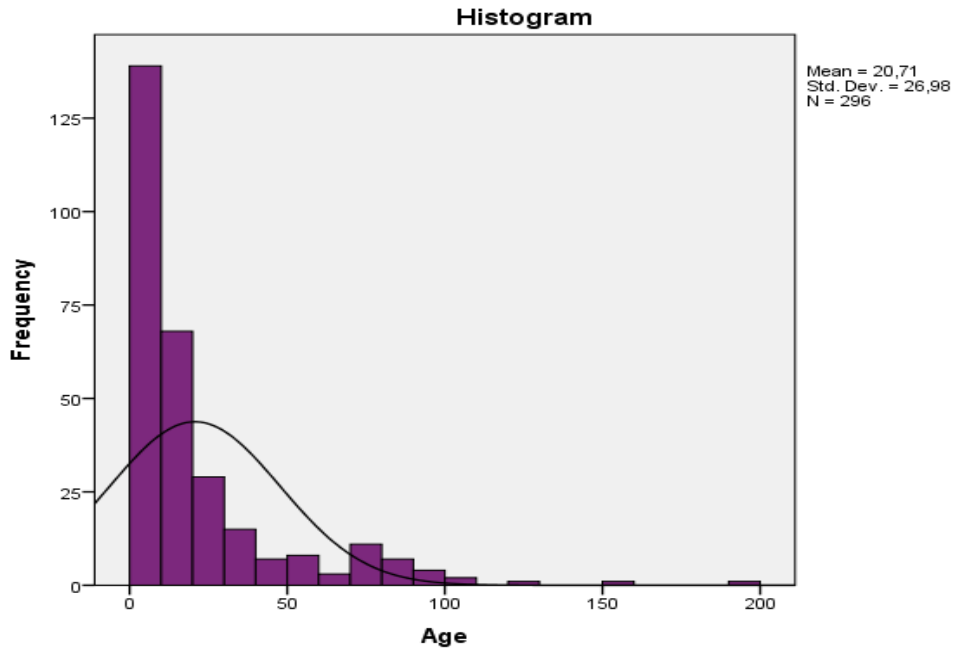


Fig. 2 Distribution by age

TABLE I

The t test for independent samples to compare IgE and the place of residence means

		Immunoglobuline E	
		Equal vari- ances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F	12,948	
	Sig.	,000	
t-test for Equality of Means	t	-,980	-,975
	df	294	281,978
	Sig. (2-tailed)	,328	,330
	Mean Difference	-,099	-,099
	Std. Error Difference	,101	,102
	95% Confidence Interval of the Difference	Lower	-,299
		Upper	,100

Babies born by cesarean section are not more likely to develop AD compared to those born naturally (9, 10, 11, 12, 13). In our study, 65.9% of children were born naturally and 34.1% by caesarean section, average of 1.34 and SD = 0.47. T test, bivariate correlation it-s not statistical significant to support the hypothesis that cesarean delivery increases the risk for developing atopy for $t(294) = -0.780$, $p > 0.05$, and F test did not reject the null hypothesis.

The frequency of food-allergy among infants and young children, which contributes to worsening of AD lesions is of approximately 20-40%. Early feeding with powdered or cow milk, early introduction of allergenic foods such as fish, egg, peanuts, in the diet of both breastfeeding mother and infant can aggravate AD lesions (6, 14, 15).

In our study group we could see how the

introduction of artificial feeding with milk powder or cow milk during infancy causes shortly after the occurrence of AD lesions. Artificially fed were 76% of children and 24% were breastfed, with a mean of 1.76 and SD = 0.42. 52.36% of cases were clinically diagnosed with cow milk protein allergy, children aged 1 month to 3 years. Determination of specific IgEs revealed: 6.08% of cases with positive specific IgE to milk, 2.36% to house dust mites, 1.68% each to egg, peanuts and animal hair and epithelia (dog, cat), 1.01% to nuts, soy, potato, fish, fruits, chocolate, and grasses. Upper gastrointestinal endoscopy was performed in one case and found eosinophilic esophagitis.

To determine if nutrition affects AD t test was used. For $t(294) = 1.30$ we obtained a $p > 0.05$ which is not statistically significant, but F test rejected the null hypothesis (tab. II).

TABLE II
Independent samples t test to compare the averages for the type of food and IgE

		Immunoglobuline E	
		Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F	10,997	
	Sig.	,001	
t-test for Equality of Means	t	1,302	1,415
	df	294	136,056
	Sig. (2-tailed)	,194	,159
	Mean Difference	,154	,154
	Std. Error Difference	,118	,109
	95% Confidence Interval of the Difference	Lower	-,079
		Upper	,387

When comparing the feeding and SCORAD means, p was greater than 0.05 and no correlation between them was found.

The estimation of borderline IgE means showed a stronger correlation between artificial feeding and natural delivery, but feeding and natural delivery also influence

atopy to a certain degree (fig. 3).

Family aggregation has been studied from the viewpoint of a family history of asthma, allergic rhinitis, atopic dermatitis and urticaria. In 82.77% of our cases we found the absence of allergies; allergies were present in 14.53% in one parent, grandparent or sibling

and in 2.70% in both parents or a sibling and a parent, with an average of 0.20, variance of 0.21 and SD = 0.46.

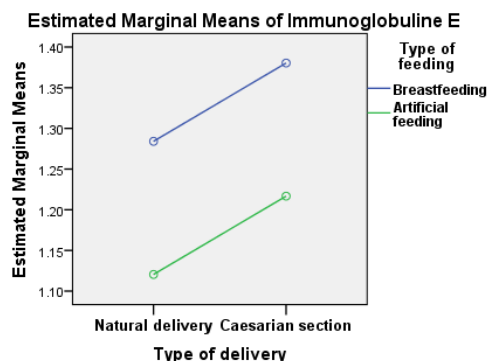


Fig. 3 Correlation between IgE feeding and type of delivery

The use of bivariate analysis showed statistically significant values for IgE, $p < 0.01$, t test for comparing means was statistically insignificant for $t(49) = 1.34$, $p > 0.05$, the null hypothesis being rejected by F test which is statistically significant at $p < 0.01$ (tab. III). One-way ANOVA of the means showed difference only between the means for both parents allergic and a brother and one parent allergic without family history of allergies, 1.75 and 1.15, respectively. The difference was not statistically significant because $p > 0.05$. Multiple comparison revealed no statistically significant differences as $p > 0.05$, even if the difference between the means was negative.

TABLE III
Independent Samples t Test to compare averages between IgE and familial history of allergy

		Immunoglobuline E	
		Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F	10,301	
	Sig.	,002	
	t	-1,344	-2,019
	df	49	17,803
	Sig. (2-tailed)	,185	,059
t-test for Equality of Means	Mean Difference	-,424	-,424
	Std. Error Difference	,316	,210
	95% Confidence Interval of the Difference	Lower	-,866
		Upper	,018

According to AD severity score, 19.94% of the children had a mild form of disease, 69.59% moderate and 10.47% severe, with an average of 2.00, SD = 0.54 and variance of 0.29.

Bivariate correlation of the means showed a strong association between IgE level and the degree of AD lesions severity for $p < 0.01$. One-way ANOVA revealed

statistically significant means differences between patients with mild and severe disease according to SCORAD index - 0.93 versus 1.35, at multiple comparison the difference of means being significant for $p < 0.05$ (tab. IV). Also the graphical representation of means shows the correlation between SCORAD score and IgE level (fig. 4).

TABLE IV

OneWay Anova to compare means between IgE and SCORAD

Immunoglobuline E					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5,977	2	2,988	4,016	,019
Within Groups	218,047	293	,744		
Total	224,024	295			

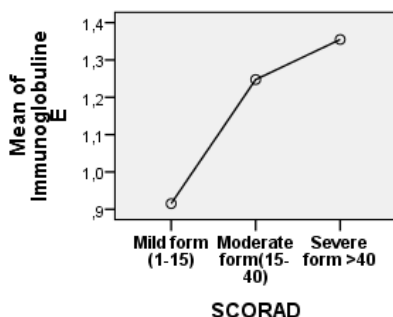


Fig. 4 Graphic representation of IgE and SCORAD means

Skin barrier impairment facilitates superinfections, especially by *Staphylococcus aureus*, *Streptococcus pyogenes*, *Herpes simplex*, *Molluscum contagiosum*. It preserves skin inflammation with further persistence of lesions (10, 17, 18). In our study skin superinfection was found in the severe forms of DA. Thus, 9 of the 31 children with severe SCORAD score presented superinfection by *S. aureus*. Of these children one each associated *Haemophilus influenzae*, *E. coli*, *P. aeruginosa*. Other colonizations: one child by *Streptococcus pyogenes* and one child by *Enterobacter spp* and *Enterococcus faecalis*.

DISCUSSIONS

The epidemiology of AD is important for identifying the risk factors that can be acted upon, it being a dynamic disease influenced by environmental and genetic factors. It is common in infants and small children. This study on AD ethiopathogen-

esis was in agreement with other similar studies by the incidence close to that in the literature and the onset most frequently occurring in infancy at the age of 2 months (10). Gender distribution was equal.

The place of residence influences the development of AD, with a higher risk in urban areas in developed countries (9, 10, 11). Given the small difference between urban and rural patients in our study, it is more likely that other factors influence the development of AD, such as processed foods or genetic background.

In our study delivery type did not increase the risk of developing AD, but some studies confirmed high birth weight and prematurity by impaired skin barrier function as risk factors (11).

Food allergies are common among patients with AD. Given the age of onset, feeding plays an important role. Breast-feeding is a protective factor against many illnesses. In AD this hypothesis is not supported by studies, as there may be variations in breast milk composition or in other factors that may influence breastfeeding. Thus, in our study the means for IgE, diet type delivery type showed that feeding and natural birth can influence atopy (6, 14, 15). Additional administration of hydrolyzed cow milk formulas and probiotic supplementation may have a beneficial effect in preventing disease development in high-risk children not exclusively breastfed (5, 10, 11, 14, 15).

The well-known risk factors that are strongly correlated with the development of DA are family history of atopy and filaggrin loss-of-function mutations. In our study we found that family history of atopy is statistical significant (11).

As currently there is no specific biological marker that differentiates between AD and other allergic diseases, the diagnosis of DA is clinical, based on history, morphology and distribution of skin lesions, and associated clinical signs (8, 10). The most common laboratory finding in AD is an elevated total IgE and/or specific IgE level. According to IgE level, researchers classified AD into two groups of disease: intrinsic and extrinsic. In our study the extrinsic group type was prevalent. Inflammatory and cytokine profile differentiate between acute onset and chronic forms. These forms are controversial because the individual with AD can later develop elevated IgE levels and specific IgE positivity as a sec-

ondary phenomenon. Moreover, although statistically the severity of AD correlates with elevated IgE levels, this does not mean that in all forms of severe AD IgE level is elevated (10, 11, 16, 18).

CONCLUSIONS

The rise in the number of AD cases requires a more thorough analysis of the way environmental factors interact with predisposition of the child to atopy.

In this case feeding and lifestyle influence the child since infancy.

Discovering the cause of this condition to reduce disease severity is difficult in many cases.

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REFERENCES

1. Johansson SGO, Hourihane JO'B, Bousquet J et al. A revised nomenclature for allergy. *Allergy* 2001; 56: 813-824.
2. Johansson SGO, Bieber T, Dahl R et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization 2003. *J Allergy Clin Immunol* 2004; 11: 832-836.
3. Just J. Histoire naturelle de la dermatite atopique: expérience des cohortes néonatales. *REV FR ALLERGOL* 2012; 52: 186-174.
4. Novak N, Leung DYM. Advances in atopic dermatitis. *Curr Opin Immunol* 2011; 23(6): 778-783.
5. Lien TY, Goldman RD. Breast feeding and maternal diet in atopic dermatitis. *Child Health Update* 2011; 57: 1403-1405.
6. Mailhol C, Giordano-Labadie F, Ammoury A, Lauwers-Cances V, Paul C, Rancé F. Dermatite atopique de l'enfant : quand penser à l'allergie alimentaire? *REV FR ALLERGOL* 2011; 50: 186-174.
7. [www.http://adserver.sante.univ-nantes.fr/Scorad_Course/Clinical_evaluation.html](http://adserver.sante.univ-nantes.fr/Scorad_Course/Clinical_evaluation.html)
8. Akdis CA, Akdis M, Bieber T et al. Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/ American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. *J Allergy Clin Immunol* 2006; 118 (1): 152-169.

9. Jochen Schmitt J, Langan S, Deckert S et al. Assessment of clinical signs of atopic dermatitis: A systematic review and recommendation. *J Allergy Clin Immunol* 2013; 132 (6): 1337-1347.
10. Schram ME, Tedja AM, Spijker R, Bos JD, Williams HC, Spuls PI. Is there a rural/urban gradient in the prevalence of eczema? A systematic review. *Br J Dermatol* 2010; 162: 964-973.
11. Zeiger RS, Heller S. The development and prediction of atopy in high-risk children: Follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. *J Allergy Clin Immunol* 1995; 95 (6): 1179-1190.
12. Eichenfield LF, Tom WL, Chamlin SL et al. Guidelines of care for the management of atopic dermatitis, Section 1. Diagnosis and assessment of atopic dermatitis. *J AM ACAD DERMATOL* 2013; 70 (2): 338-351.
13. Rudikoff D, Cohen SR, Scheinfeld N. *Atopic dermatitis and eczematous disorders*. Boca Raton, London, New York, CRC Press Taylor&Francis Group 2014.
14. Conde-Taboada A, González-Barcala FR, Toribio J. Review and Update of Current Understanding of Childhood Atopic Dermatitis. *Actas Dermosifiliogr*. 2008; 99:690-700.
15. Panduru M, Panduru NM, Ion DA. Caesarean section delivery and atopic dermatitis – meta-analysis of observational studies. *Gineco Ro* 2012; 8 (30): 196-198.
16. Greer FR, Sicherer SH, Wesley Burks A, the Committee on Nutrition and Section on Allergy and Immunology. Effects of Early Nutritional Interventions on the Development of Atopic Disease in Infants and Children: The Role of Maternal Dietary Restriction, Breastfeeding, Timing of Introduction of Complementary Foods, and Hydrolyzed Formulas. *PEDIATRICS* 2008; 121 (1): 183-191.
17. Sicherer SH, Sampson HA. Food hypersensitivity and atopic dermatitis: Pathophysiology, epidemiology, diagnosis, and management. *J Allergy Clin Immunol* 1999; 104 (3): 114-122.
18. Bin L, Kim BE, Brauweiler A et al. Staphylococcus aureus a-toxin modulates skin host response to viral infection. *J Allergy Clin Immunol* 2012; 130 (3): 683-691.
19. Rugină A, Rusu T, Ioniuc I. Comorbidităţi alergice în astmul bronşic la copil. *Rev. Med. Chir. Soc. Med. Nat., Iaşi* 2010; 114 (3): 633-637.

NEWS

HORMONAL CONTRACEPTIVES, GENITAL TRACT INFECTIONS AND HIV RISK

The hormonal contraceptives and genital tract infections are associated with altered cervical immunity. A recent study that evaluated changes in cervical immunity after taking hormonal contraceptives showed that the women who used injectable depot medroxyprogesterone acetate and those who use estrogen-progesterone oral contraceptives had increased levels of proteins that attract HIV host cells. Also, the effect of hormonal contraceptives was exacerbated in presence of disturbed vaginal microbiota or genital tract infections such as gonorrhea, chlamydia, genital herpes, trichomoniasis and candidiasis. The levels and the type of immune mediators, infection status and hormonal contraceptives increase HIV risk by their cumulative action. (Fichorova RN, Chen P-L, Morrison CS, Doncel GF, Mendonca K, Kwok C, Chipato T, Salata R, Mauck C. 2015. The contribution of cervicovaginal infections to the immunomodulatory effects of hormonal contraception. *mBio* 6(5):e00221-15. doi:10.1128/mBio.00221-15).

Cătălina Luncă

ASSESSMENT OF ADVERSE DRUG REACTIONS AS CARDIOVASCULAR RISK FACTORS

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ASSESSMENT OF ADVERSE DRUG REACTIONS AS CARDIOVASCULAR RISK FACTORS (Abstract): A wide range of pharmaceutical substances can induce side-effects expressed as cardiovascular changes or events, adding to other risk factors or worsening preexisting cardiovascular diseases. **Aim:** Continuous study focused on iatrogenic conditions representing cardiovascular risk factors. **Methods:** We developed a descriptive study of patients admitted to the Iasi Vth Internal Medicine and Geriatrics-Gerontology Clinic between 1998-2013, focusing on iatrogenic conditions representing cardiovascular risk factors. **Results:** We have diagnosed 81 cases of drug-induced hypertension, and 43 patients with hypertensive crises; 72 cases of iatrogenic hyperglycemia; 36 cases of drug-induced hyperuricemia; 50 cases of drug-induced dyslipidemias; and 17 cases of iatrogenic obesity. These iatrogenic diseases were more common in women and the elderly. Twenty-eight patients have developed simultaneous adverse drug reactions induced by the same drug and manifest as different cardiovascular risk factors. **Conclusions:** Cardiovascular risk factors can be induced to a significant extent by chronic drug administration. Some medications (e.g. NSAIDs, corticoids, β -blockers, diuretics, contraceptives) can act on the same patient by multiple pathogenic links. The adverse drug reactions can be cardiovascular risk factors that persist in time, or can be removed (by discontinuing the administration of the implicated drug). The highest importance of their acknowledgment relies on the possibility of their prevention through carefully balancing the benefits and the risk of each new medication. **Keywords:** DRUG-INDUCED SIDE-EFFECTS, CARDIOVASCULAR DISEASES, CARDIOVASCULAR RISK FACTORS, IATROGENESIS.

World Health Organization defines pharmacovigilance as "the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem". The importance of pharmacovigilance lies in its key place in the public

health care system (1).

A wide range of pharmaceutical substances can induce side-effects expressed as cardiovascular events, adding to other risk factors or worsening preexisting cardiovascular diseases. In countries with a very rigorous pharmacovigilance activity, such

as the Netherlands and UK, these cardiovascular iatrogenic effects reach up to 10% of all annual reported iatrogeneses (2). The most frequently found are heart rhythm disturbances, heart failure, high blood pressure, myocardial ischemia, thromboembolic disease, myocardial toxicity, valvular and pericardial heart disease (3,4,5).

Life-threatening adverse drug reactions (ADR) reports were submitted by patients in a range of 8.6% in Denmark up to 13% in Netherlands (2,6,7).

MATERIAL AND METHODS

We developed a continuous study aimed at identifying iatrogenic conditions representing cardiovascular risk factors in a population of hospitalized patients. Thus, we searched for iatrogenic hypertension, diabetes, dyslipidemia, obesity and hyperuricemia that might have occurred before admission or during hospital stay.

The observational study recorded the adverse reactions occurring in patients admitted between 1998-2013 to the Iasi Vth Internal Medicine and Geriatrics-Gerontology Clinic of the “Railway Hospital”. The studied population consisted of 18,740 patients, of which 8,900 (47.49%) women and 9,840 (52.51%) men.

In order to establish the possibility of a drug side-effect the classical criteria of incrimination used in pharmacovigilance were used (8). Besides this intensive surveillance, some other clinical trials were designed in order to determine if the first assumptions are supported.

The study continued with a follow up of these patients during current and second admission for either an acute event or reassessment of their health status. One hundred ninety-nine patients only benefited from outpatient consultation.

RESULTS

1496 (7.98%) of the patients presented 1677 different types of adverse drug reactions (some of the patients experiencing more side effects) with different degrees of severity. Out of them 243 patients (16.24%) presented 299 adverse drug reactions that can be considered cardiovascular risk factors: hypertension, diabetes, dyslipidemia, obesity and hyperuricemia.

Age and sex distribution of the 299 cases with adverse reactions is shown in fig. 1 (some patients manifested multiple side effects) (fig. 1) and they were statistically analyzed (Data_cases of pharmacovigilance.xls).

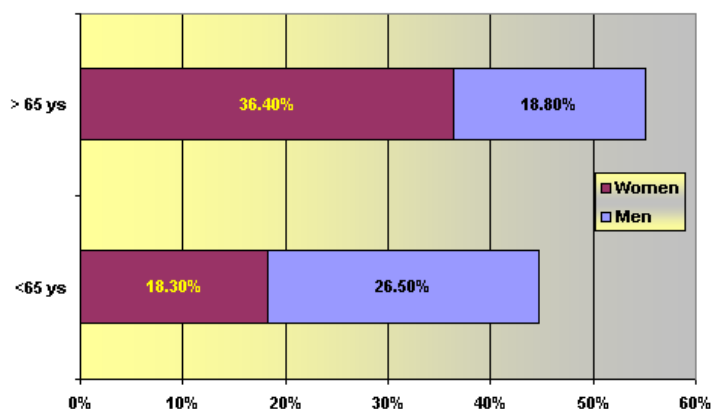


Fig. 1. The age and sex distribution of adverse drug reactions

The recorded iatrogenic effects were found in patients with cardiovascular diseases on long-term or short-term medication administered to treat different conditions or to reduce multiple risk factors (tab. I).

TABLE I
Adverse drug effects caused by the incriminated drugs

Adverse drug reaction	Incriminated drug	No of cases
Hypertension	Nonsteroidal anti-inflammatory drugs (NSAIDs)	25
	Oral contraceptives	17
	Corticosteroids	14
	Monoamine oxidase inhibitors (MAOIs)	7
	β -mimetics	5
	Thyroid hormones	5
	Anabolic steroids	5
	α -interferon	3
Hypertensive crises	Beta-blocker withdrawal syndrome	12
	NSAIDs	20
	Thyroid hormones	11
Diabetes mellitus/ impaired fasting glucose and/or impaired glucose tolerance	Diuretics (thiazide, furosemide, indapamide)	26
	Oral contraceptives	12
	Oral corticosteroids	9
	NSAIDs	5
	Atypical antipsychotic medications (olanzapine)	5
	β -blockers	4
	Calcium antagonists	3
	Nalidixic acid	3
	Intra-articular administration of corticosteroids	2
	Ciprofloxacin	2
	Propafenone	1
	Statin	1
Hyperuricemia	Diuretics (thiazide, indapamide)	25
	Salicylates	7
	Phenylbutazone	3
	Ranitidine	1
Dyslipidemias	Diuretics (thiazide)	17
	Oral contraceptives	11
	Corticosteroids	8
	Atypical antipsychotic medications (olanzapine)	8
	β -blockers	5
	Angiotensin -converting enzyme inhibitors	1
Obesity	Cortisone	6
	Atypical antipsychotic medications (olanzapine)	6
	Anabolic steroids	4
	Statin	1

Drug-induced hypertension was found as an adverse drug reaction in 81 cases (33.33%), more frequently involved being the nonsteroidal anti-inflammatory drugs (NSAIDs), oral contraceptives and corticosteroids. Monoamine oxidase inhibitors (MAOIs), β -mimetics, thyroid hormones, anabolic steroids or α -interferon were also incriminated in drug-induced hypertension, but with a lower frequency.

In agreement with other authors (9,10), we found neither specific clinical criteria for drug-induced hypertension (patients presenting with a wide range of clinical manifestations) nor temporal criteria (the onset as well as the time to the normalization of blood pressure in case of withdrawal being highly variable). In most of the cases with hypertension caused by NSAIDs and oral contraceptives (which were the leading cause), patients had taken the medication without medical prescription, and did not follow the instructions (low sodium diet).

The rise in blood pressure was usually mild, but 11 cases (4.53%) developed severe hypertension and discontinuing the incriminated drug led to the normalization of blood pressure within 2 weeks - 6 months.

The triggering of hypertensive crisis in 43 patients (17.70%) already known with high blood pressure was recorded in 3 situations: withdrawal syndrome following cessation of antihypertensive drug therapy (the abrupt discontinuation of β -blocker treatment) in 12 cases (4.94%), higher blood pressure levels associated with NSAIDs administration confirmed by 24-hours arterial blood pressure measurements in 20 patients (8.23%), and hypertensive episodes in 11 patients (4.53%) on thyroid hormone replacement therapy.

Diabetes mellitus was the second most frequent cardiovascular adverse effect.

A correlation between the administration of a drug and the occurrence of hyperglycaemia was found in 72 patients (29.63%). In 58 cases (23.87%), the incrimination of the drug was very likely. The drugs most frequently involved were diuretics (thiazide, frusemide, indapamide), oral contraceptives and oral corticosteroids, followed by NSAIDs and atypical antipsychotic medications (olanzapine). Some patients had a history of chronic treatment for such diseases as tuberculosis (isoniazid and rifampicin), or persistent lumbar pain (NSAIDs). In a small number of patients we have noticed the involvement of amiodarone, statin, and atypical antipsychotic medication (olanzapine) in the onset of diabetes mellitus.

The relationship between drug use and hyperglycemia was weaker (possible or likely reaction) in 10 cases, and was related to β -blockers, calcium antagonists and nalidixic acid. Intra-articular corticosteroids, ciprofloxacin and propafenone produced this side effect with a lower frequency.

Of the drugs with hyperglycemic effect, only those well-known to induce this metabolic effect were most likely incriminated, but of the drugs with a smaller coefficient of incrimination (< 5), some less mentioned drugs like ciprofloxacin, or never mentioned – as propafenone – were responsible for this side-effect.

Hyperglycemic crisis, possibly secondary to drugs was found in 2 patients (0.82%) that were long-term treated with nifedipine and 1 patient receiving verapamil on long-term; if this side-effect of nifedipine is already known, the role of verapamil is controversial, some authors advocating

its hyperglycemic role or pleading for its hypoglycemic role, while others support its neutral role on the carbohydrate metabolism (11,12).

Drug-induced hyperuricemia (or iatrogenic hyperuricemia) (13) was found in 36 cases (14.81%) and was mainly caused by chronic diuretic use, as mentioned above, in 25 cases (10.29%). In other 7 cases (2.88%), secondary hyperuricemia was due to salicylates, while the frequency of this side-effect in patients treated with ranitidine and phenylbutazone was lower.

Diuretic-induced gout occurred in 11 cases (4.53%), and it was reversible following withdrawal of the drugs in 10 cases, while in 1 case it required treatment with allopurinol.

Drug-induced dyslipidemia (14) was diagnosed in 50 patients (20.58%). In the majority of cases, diuretics and oral contraceptives, together with corticosteroids and olanzapine caused different types of secondary iatrogenic dyslipidemias.

Obesity was recorded in 17 cases (~7%) on long-term medication use. Most drug-induced weight gain effects were the result of chronic treatment with cortisone and olanzapine. In few patients obesity was associated with an adverse drug reaction to anabolic steroids or statin regimen.

Our study proves that atypical antipsychotic medication increases the likelihood of many cardiovascular risk factors, confirming the conclusion that weight gain and metabolic disease in patients taking second-generation antipsychotic medications “represents an epidemic within an epidemic” (15).

The discontinuation of suspected drugs led to normalization of clinical and biological data in less than 3 months in the acute forms and in less than 6 months in the chronic ones. 17 patients (~7%) were

lost to follow up. No rechallenge was voluntarily administered.

DISCUSSION

Patients developing drug-induced diabetes mellitus or hyperglycemia were not previously known with diabetes mellitus or with abnormal glucose tolerance, although, theoretically, diabetics are more prone to develop this side-effect. On the other hand, the acknowledgement of the hyperglycemic effect of some drugs makes doctors to avoid their usage in diabetics.

We have found no causal relationship between the corticosteroid dose and the occurrence of hyperglycemia in non-diabetic patients; conversely, an increased likelihood of corticosteroids dose-related hyperglycemia was found in diabetics.

All patients with oral or intra-articular administration of corticosteroids were diabetics, and hyperglycemia occurred when, for various reasons, the patient has been given a higher dose than the initial one.

Statin-induced hyperglycemia was described in a 72-year-old woman, who also exhibited muscle weakness upon standing and walking, particularly in the quadriceps muscles, within 15 days of treatment for hypercholesterolemia. After statin administration was discontinued, blood sugar level returned to normal within 5 days, but, nevertheless, the patient was still complaining of muscle soreness and decreased exercise tolerance for another month. The cardiovascular risk might be augmented by the imposed sedentary behaviour. Symptoms had a lower intensity after one month, when she went to the ambulatory care clinic, and the caregiving team reassured and encouraged her to practice daily limb exercise training and walking.

A higher frequency of iatrogenic diseases was found in women, which general-

ly consume more frequently over-the-counter medication such as NSAIDs, diuretics, contraceptives. The long-term medication that led to the occurrence of adverse drug reactions was self-medication in 49 cases (20.16%), out of which almost 71% (35 cases) were women.

The higher frequency of iatrogenic disease in the elderly is caused by age-related alterations in pharmacokinetics and pharmacodynamics, polypragmasia (due to polypathology at this age), predisposition to many unwanted interactions and difficult cooperation with the old patient (16,17).

The importance of drugs as cardiovascular risk factors relies not only in their frequency (lower than other etiologies), but especially in the fact that they represent modifiable risk factors (therefore they are avoidable by ceasing treatment, if the administration is not absolutely necessary). It is also important to acknowledge the possibility that the same drug may simultaneously induce multiple risk factors (e.g.: corticosteroids, NSAIDs, β -blockers, diuretics, contraceptives) (18). The adverse drug reactions proved to be cardiovascular risk factors that disappeared within several days to several weeks after withdrawal of suspected drug and persisted for months only in three cases of obesity.

The more frequent occurrence of these iatrogenic effects in older women, as proved by this study, requires an increased focus on this segment of the population (considering that they additionally lose cardiovascular protection due to the drop in

estrogen level associated with menopause).

Our study describes the following paradox: trying to treat cardiovascular risk factors may cause other cardiovascular risk factors. Moreover, a patient receiving multiple medication in order to avoid the occurrence of cardiovascular disease may become noncompliant, ignoring the effects of diet and physical exercise. It is the physician's role to inform the patient on the possible negative effects of polypragmasia and the beneficial role of a healthy lifestyle (19,20).

CONCLUSIONS

1. The study proved that cardiovascular risk factors can be induced to a significant extent by chronic drug administration.

2. Some drugs (NSAIDs, β -blockers, diuretics, contraceptives) cause simultaneous cardiovascular adverse reactions in the same patient by multiple pathogenic links.

3. The adverse drug reactions disappeared within several days to several weeks after the discontinuation of the suspected drug and rarely persisted for months.

4. The highest importance of their acknowledgment relies on the possibility of their prevention through carefully balancing the benefits and risk of each new medication.

5. The adverse reactions can be counteracted by healthy lifestyle interventions, comprising balanced diet and regular exercise training, habits that can be enhanced through counselling and continuous medical education.

REFERENCES

1. World Health Organization. *The Importance of Pharmacovigilance* 2002; WHO/EDM/QSM/2002.2
2. Danish Health and Medicines Authority. Adverse drug reaction reported by consumers in Denmark – compared with reports from healthcare professionals. 2013; <http://www.sst.dk/english>

3. Lai KN, Richards AM, Nicholls NG. Drug-induced hypertension. Adverse Drug Reaction. *Acute Toxicol Rev* 1991; 10: 31-46.
4. Rojdmarm S, Andersson D. Influence of verapamil on human glucose tolerance. *Am J Cardiol* 1986; 57: 39D-43D.
5. Andrejaka M, Tribouilloy C. Drug-induced valvular heart disease: An update. *Arch Cardiovasc Dis* 2013; 106: 333-339.
6. Davies DM. *Textbook of Adverse Drug Reactions*. Oxford University Press, 1991.
7. Avery AJ, Anderson C, Bond CM, et al. Evaluation of patient reporting of adverse drug reactions to the UK 'Yellow Card Scheme': literature review, descriptive and qualitative analyses, and questionnaire surveys. *Health Technol Assess* 2011; 15(20): .
8. Benichou C. Management of adverse events during clinical trial. In: Benichou C, ed. *Adverse Drug Reaction. A Practical Guide to Diagnosis and Treatment*. Chichester, UK: John Wiley and Sons Ltd. 1994:223-232.
9. MacLean F, Lee A. Drug-induced cardiovascular disorders. *Pharm J* 1999; 262(7029): 113-118.
10. Grossman E, Messerli FH. Drug-induced Hypertension: An unappreciated cause of secondary hypertension. *Am J Med* 2012; 125(1): 14-22.
11. Luna B, Feinglas MM. Drug-induced hyperglycemia. *JAMA* 2001; 286: 1945-1948.
12. Chan NN, Osaki R, Chaw CC. Drug-related hyperglycaemia. *JAMA* 2002; 287(6): 2001-2014.
13. Hunter DJ, York M, Chaisson CE, et al. Recent diuretic use and the risk of recurrent gout attacks: the online case-crossover gout study. *J Rheumatol* 2006; 33:1341-1345.
14. Vodnala D, Rubenfire M. Secondary causes of dyslipidemia. *Am J Cardiol* 2012; 110(6): 823-826.
15. Fenton WS, Chavez MR. Medication-Induced Weight Gain and Dyslipidemia in Patients With Schizophrenia. *Am J Psych* 2006; 163: 1697-1704.
16. Alexa ID, Ilie AC, Morosanu A, Voica A. Approaching frailty as the new geriatric syndrome. *Rev. Med. Chir. Soc. Med. Nat. Iași* 2013; 117(3): 680-685.
17. Ungureanu G, Alexa ID, Stoica O. Iatrogenia si varstnicii. *Rev. Med. Chir. Soc. Med. Nat. Iași* 2007; 3(4): 803-810.
18. Chansan-Taber L, Stampfer MS. Epidemiology of oral contraceptives and cardiovascular diseases. *Ann Intern Med* 1998; 128: 467-477.
19. Azoică D, Manole A, Trifan M. *Ghid pentru pregătirea în asistența primară a stării de sănătate și epidemiologie*. Editura „Gr.T.Popa” Iași 2004.
20. Leon MM, Mitu F. The clinical epidemiological study of drugs used to treat arterial hypertension and comorbidities associated. *Rev. Med. Chir. Soc. Med. Nat. Iași* 2013;117(2):488-494.

PLATELET INDICES AND LIVER FIBROSIS EVALUATION IN CHRONIC HEPATITIS C

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PLATELET INDICES AND LIVER FIBROSIS EVALUATION IN CHRONIC HEPATITIS C (Abstract): Recently, several studies have reported that the mean platelet volume and platelet distribution width may give information about liver fibrosis severity in chronic hepatitis C. **The aim** of the present study was to evaluate whether platelet indices correlate with hepatic fibrosis measured by transient elastography in patients with chronic hepatitis C. **Materials and methods:** Patients with chronic hepatitis C were prospectively enrolled. Samples for complete blood count and routine biochemical parameters were collected and analyzed in the same day with liver fibrosis assessment by transient elastography. Mean platelet volume, platelet large cell ratio and platelet distribution width were compared with stages of liver fibrosis. Statistical analysis was carried out using SPSS 17.0 software. A P-value of less than 0.05 was considered statistically significant. **Results:** There were 139 patients with chronic hepatitis C (70.5% males, mean age 54.8 ± 16.7 years). Compared with mild/moderate liver fibrosis patients, those with advanced liver fibrosis had an increased mean platelet volume (10.4 ± 0.7 vs. 10.9 ± 0.9 , $p < 0.002$), platelet large cell ratio (28.5 ± 5.3 vs. 32.5 ± 7.2 , $P < 0.0001$), and platelet distribution width (12.8 ± 1.5 vs. 14.1 ± 2.7 , $P = 0.003$). **Conclusions:** Increased platelet indices were associated with advanced liver fibrosis stages evaluated by transient elastography in patients with chronic hepatitis C. **Keywords:** PLATELET INDICES, LIVER CIRRHOSIS, HEPATITIS C, FIBROSIS, TRANSIENT ELASTOGRAPHY.

Today, chronic hepatitis C virus (HCV) infection affects more than 180 million individuals worldwide, being among the world's leading causes of death and the major reasons for the increasing prevalence of liver cirrhosis and hepatocellular carcinoma (1).

Staging of liver fibrosis is important for the estimation of prognosis, for surveillance and for treatment decisions in patients with HCV. Liver biopsy is still con-

sidered “gold standard” for the staging of liver fibrosis in spite of its several shortcomings such as intra- and inter-observer variability in histopatologic interpretation, sampling errors, variable accessibility, and high cost; in addition, it is an invasive procedure, sometimes associated with rare but potentially life-threatening complications (2,3). Therefore, noninvasive methods such as serum fibrosis markers and ultrasound-based elastography have been inten-

sively evaluated for the assessment of liver fibrosis over the past two decades and many of them have proven to be excellent tools for staging fibrosis in patients with chronic HCV (4). Serum fibrosis markers may be used singly (5) or in combination, some of which are patent-protected and commercially available: FibroTest® (6), Fibrometer® (7), ELF® (8), and Hepascore® (9), with no risk for the patient and no contraindication, with interlaboratory reproducibility. The advantage of serum markers is that they are largely available and reproducible; the disadvantage is that they can be influenced by the comorbidities and medications. A simple, cost-free, readily available test in all hospital setting is APRI (AST/platelets ratio) (10). Transient elastography (TE) measures liver stiffness and has highest level of performance among noninvasive methods for diagnosis of advanced fibrosis and cirrhosis (11).

Over the last few years, several studies have evaluated the platelet indices for the assessment of liver fibrosis in patients with chronic hepatitis B virus (HBV) (12,13) and HCV (14). Platelet indices include mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-LCR). Some studies have reported that MPV and PDW may give information about liver fibrosis severity in HBV and HCV (12-14), although the relationship between these and other platelet indices and liver fibrosis is still controversial (13,15). Recently, Purnak et al. demonstrated that increased MPV is an independent predicting factor for liver fibrosis in patients with chronic HCV (14). However, to our knowledge, the association between platelet indices and liver fibrosis in hepatitis C virus related liver disease remains mainly unknown.

The aim of the present study was to evaluate whether platelet indices correlate with hepatic fibrosis measured by TE in patients with chronic HCV.

MATERIAL AND METHODS

One hundred and thirty-nine patients with known chronic HCV naïve to antiviral treatment and followed-up in a tertiary care center, who have undergone TE examination from January 2013 to June 2014 were prospectively enrolled in this study. Exclusion criteria involved all conditions that are known to modify platelet indices such as chronic liver diseases other than HCV, type 2 diabetes mellitus, hypertension, hypercholesterolemia, obesity, malignancy, and hypochromic anemia (16,17). None of the patients included have received anticoagulant or antiaggregant medications, non-steroidal antiinflammatory drugs (NSAID) and oral contraceptives before hospital admission because all these drugs could influence platelet aggregability.

The study protocol was approved by the local Ethics Committee, and all patients were consecutively enrolled to the study after providing written informed consent.

Liver fibrosis assessment. All patients had liver stiffness measured by TE (FibroScan®, Echosens, Paris, France). At least 10 valid measurements, a 60% success rate, and an interquartile range of less than 30% of the median elasticity were required for a measurement to be valid. The liver stiffness was expressed in kiloPascal (kPa), and values range from 2.5 to 75 kPa, with normal values <5.5 kPa (18). According to Metavir score, a cutoff value of 8.7 kPa defines mild (5.6-7.1 kPa=F1) or moderate (7.2-8.7 kPa=F2) fibrosis, while values ≥ 8.7 kPa defines advanced fibrosis (8.8-12.5 kPa=F3; ≥12.5 kPa=F4) [19]. In this study

patients were divided in two groups: mild/moderate fibrosis (F1-F2) and advanced fibrosis (F3-F4).

Serum measurements. Samples for complete blood count analysis were obtained by phlebotomy using minimal pressure and collected into a vacutainer tube, containing ethylenediaminetetraacetic acid (EDTA). These blood samples were analyzed within 2 h on automatic blood counter Sysmex XT-4000i (Kobe, Japan). All tests were performed according to the standardized protocols recommended by the manufacturers (17).

For the determination of platelet indices we used an automated hematology analyzer that could overestimate platelet count especially in thrombocytopenic samples compared with the immunological reference method. The platelet indices were derived from the platelet distribution curve obtained from impedance method. It includes MPV that describes the average platelet size reported in femtolitre (fL), PDW defined as the distribution width at 20% frequency level calculated as the standard deviation of platelet volume divided by MPV, and measures the heterogeneity in platelet size. Derived platelet indices are highly specific to the individual technologies, with different analyzers having different reference ranges. As separate feature, the platelet large cell ratio, P-LCR, can be reported specifically on Sysmex analyzers and is the percentage of platelet larger than 12 fL.

Statistical analysis was carried out using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as median and interquartile ranges and categorical data as percentage. Mann-Whitney U test was used to compare groups, with the chi-square test used for

categorical variables. A P value of less than 0.05 was considered statistically significant.

RESULTS

Patient characteristics. In the study period there were admitted 658 patients with chronic liver disease, 226 (34.4%) were diagnosed with HCV, and 139 (61.5%) met the inclusion criteria. There was no difference regarding age, sex or severity of liver disease between patients included and those excluded from the study.

Clinical and biochemical characteristics of the patients are summarized in tab. I. There were 139 patients with chronic HCV (70.5% male, mean age 54.8 ± 16.7 years).

The fibrosis spectrum in patients with chronic HCV was as follows: 25 (17.9%) with stage 1, 39 (28.1%) with stage 2, 15 (10.8%) with stage 3, and 60 (43.2%) with stage 4. All the patients with stage 4 liver fibrosis had compensated liver disease, Child-Pugh class A. Of these 9 (15%) were diagnosed with esophageal varices.

Significant increases in serum alanin aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma-glutamyl transpeptidase, and significant decreases in albumin, hemoglobin, leucocytes and platelet count were observed in patients with advanced fibrosis (F3-F4).

Comparison of platelet indices between the study groups. There was a significant difference regarding the MPV, PDW and P-LCR values between the patients with mild/moderate fibrosis and those with severe fibrosis ($P=0.002$, $P=0.003$, $P<0.0001$). Platelet counts were significantly lower in patients with advanced fibrosis compared with those with mild/moderate fibrosis (240.8 ± 52.1 vs. 156.7 ± 75.8 , $P<0.0001$) (tab. II).

TABLE I
Baseline clinical and biochemical characteristics of study population

Variable	F1-F2 median (IQR) (n=64)	F3-F4 median (IQR) (n=75)	P
Age, yrs	58 (31)	65 (16)	0.002
Leucocytes/mm ³	7150 (1950)	5210 (1680)	<0.0001
Hemoglobin, g/dl	13.9 (1.7)	13.7 (2.45)	0.030
Platelets, 10 ⁹ /L	233 (76)	142 (161)	<0.0001
ALT, UI/L	24 (20)	69 (288)	<0.0001
AST, UI/L	22 (4)	65 (133)	<0.0001
Alkaline phosphatase, UI/L	84 (35)	108 (57)	<0.0001
GGT, UI/L	37 (21)	73 (108)	<0.0001
PT, sec	11.4 (1.3)	11.9 (2.05)	0.086
Albumin, g/l	4.5 (0.5)	3.5 (0.4)	<0.0001
Total bilirubin, mg/dl	0.66 (0.46)	0.74 (0.48)	0.001
CRP, mg/L	0.12 (0.17)	0.10 (0.16)	0.617
Fibrinogen, mg/dl	367 (98.75)	326 (78.25)	0.464
Spleen size, mm	114 (24)	126 (21)	<0.0001
Portal vein diameter, mm	9 (1)	13 (2)	<0.0001

ALT alanine aminotransferase, AST aspartate aminotransferase, CRP C-reactive protein, GGT gamma-glutamyl transpeptidase, IQR interquartile ranges, PT prothrombin time.

TABLE II
Platelet indices in relation to the fibrosis stages in patients with chronic HCV

Variables	F1-F2 median (IQR) (n=64)	F3-F4 median (IQR) (n=75)	F	P
MPV, fL	10.5 (0.90)	11.0 (1.7)	9.820	0.002
P-LCR, %	28.8 (6.70)	32.5 (13.90)	13.762	<0.0001
PDW, %	12.65 (2.10)	13.9 (4.75)	9.362	0.003

IQR interquartile ranges, MPV mean platelet volume, PDW platelet deviation width, P-LCR platelet large cell ratio

When all of the patients were considered in the analysis, the strongest and most inverse correlation was found between liver stiffness and platelet count ($r_s = -0.607$, $P < 0.001$), followed by serum albumin ($r_s = -0.566$, $P < 0.001$), and prothrombin time ($r_s = -0.505$, $P < 0.001$). There was also a strong correlation between liver stiffness and spleen size ($r_s = 0.504$, $P < 0.001$), followed by serum ALT, and then by total bilirubin ($r_s = 0.358$, 0.335 , respectively, $P < 0.001$).

There were no correlations between platelet indices and liver stiffness value measured by transient elastography.

DISCUSSIONS

Liver biopsy is still considered gold standard for the staging of liver fibrosis. However, its diagnostic sensitivity is limited by significant variability of the sampling and histological assessment (small sample size not representative to the entire

liver) (2,3). Furthermore, severe complication such as bleeding, hemoperitoneum, or even death could occur in some patients (20). All these disadvantages make it impractical to assess disease progression by serial biopsies in routine clinical practice (21).

Transient elastography (Fibroscan®) is reproducible, non-invasive and validated test for the assessment of liver fibrosis (11,22). Along with this imagistic method, there are also several direct and indirect markers used alone or in combination, some patent-protected, which have proven excellent methods for staging fibrosis in patients with chronic HCV and HBV (4-7). The advantages of all noninvasive methods include no risk for the patient and no contraindication, widespread availability, easy to perform and repeat in outpatient clinic and at the bedside, well accepted by the patients and thus, can be repeated for longitudinal disease monitoring which is difficult, if not impossible, with liver biopsy (22,24). However, both Fibroscan® and some patent-protected serum markers are still expensive. It was this context that assessment of platelet indices triggered much interest as possible non-invasive, widely available, practically costless serum markers of liver fibrosis in patients with chronic HBV or HCV (12-15, 25, 26). Recently, there have been published several studies on chronic HBV patients, concluding that MPV might be a marker for assessment of liver fibrosis (12). Some studies have suggested that elevated MPV levels may be an independent predictor of cirrhosis (25) or associated with liver disease activity (26) in patients with chronic HBV or portal vein thrombosis (27).

Data on platelet indices and chronic HCV are scanty. Purnak et al (14) demon-

strated that MPV is increased in patients with advanced fibrosis, and this marker along with other serological markers could give information about liver fibrosis severity in chronic HCV. The authors demonstrated that patients with HCV had significantly higher levels of MPV as compared to the control group, despite the same number of platelets, concluding that high levels of MPV could represent an indirect early marker of advanced liver fibrosis in such patients.

Our study showed that high platelet indices (MPV, PDW and P-LCR) could differentiate between mild/moderate and advanced fibrosis in HCV patients. These results confirmed some previously mentioned studies including patients with chronic HCV (14). It has been reported that increased platelet indices are directly related with thrombocytopenia, which is a direct reflection of platelet destruction by hypersplenism (28). In advanced stages of fibrosis thrombocytopenia is a multifactorial condition attributed to the sequestration of platelets by the spleen secondary to portal hypertension, reduced thrombopoietin which regulates megakaryocyte maturation and platelet production, and bone marrow suppression (28). All these mechanisms could influence platelet indices, because thrombocytopenia is not only a reflection of platelet destruction, but also of platelet production, especially in advanced chronic liver disease.

Our study has some advantages: such as being the first prospective study that evaluated the role of all platelet indices in patients with chronic HCV, using transient elastography for liver fibrosis quantification. It has also some limitations as relatively small sample size, and lack of evaluation of dynamics of platelet indices. Our

results need to be confirmed by further investigations on larger groups of patients.

CONCLUSIONS

In our study the platelet indices, MPV,

PDW and P-LCR are associated with fibrosis stage measured by transient elastography in patients with chronic HCV, and they might be used as markers to evaluate liver fibrosis stage.

REFERENCES

1. Maasoumy B, Wedemeyer H. Natural history of acute and chronic hepatitis C *Clin Gastroenterol* 2012; 26(4): 401-441.
2. Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; 38: 1449-1457.
3. Sanai FM, Keeffe EB. Liver biopsy for histological assessment: The case against. *Saudi J Gastroenterol* 2010; 16: 124-132.
4. Trifan A, Stanciu C. Checkmate to liver biopsy in chronic hepatitis C? *World J Gastroenterol* 2013; 18(39): 5514-5520.
5. Murawaki Y, Ikuta Y, Okamoto K, Koda M, Kawasaki H. Diagnostic value of serum markers of connective tissue turnover for predicting histological staging and grading in patients with chronic hepatitis C. *J Gastroenterol* 2001; 36: 399-406.
6. Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; 357: 1069-1075.
7. Calès P, Oberti F, Michalak S, et al. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005; 42: 1373-1381.
8. Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004; 127: 1704-1713.
9. Adams LA, Bulsara M, Rossi E, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005; 51: 1867-1873.
10. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38: 518-526.
11. Degos F, Perez P, Roche B, et al. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). *J Hepatol* 2010; 53: 1013-1021.
12. Ekiz F, Yuksel O, Kocak E, et al. Mean platelet volume as a fibrosis marker in patients with chronic hepatitis B. *J Clin Lab Anal* 2011; 25(3): 162-165.
13. Ceylan B, Fincanci M, Yardimci C, et al. Can mean platelet volume determine the severity of liver fibrosis or inflammation in patients with chronic hepatitis B? *Eur J Gastroenterol Hepatol* 2013; 25(5): 606-612.
14. Purnak T, Olmez S, Torun S, et al. Mean platelet volume is increased in chronic hepatitis C patients with advanced fibrosis. *Clin Res Hepatol Gastroenterol* 2013; 37(1): 41-46.
15. Tekin T. Mean platelet volume is not a predictive marker of histopathological changes of the liver in patients with chronic hepatitis B. *Clin Res Hepatol Gastroenterol* 2013; 37: e121-e123.
16. Leader A, Pereg D, Lishner M. Are platelet volume indices of clinical use? A multidisciplinary review. *Ann Med* 2012; 44: 805-816.
17. Buttarello M, Plebani M. Automated blood cell counts: state of the art. *Am J Clin Pathol* 2008; 130: 104-116.

18. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008; 48: 835-847.
19. de Ledinghen V, Vergniol J. Coreelation between liver stiffness (kPa) and fibrosis stage according to Metavir score. *Gastroenterol Clin Bio* 2008; 32: 58-67.
20. Friedman LS. Controversies in liver biopsy: who, where, when, how, why? *Curr Gastroenterol Rep* 2004; 6: 30-36.
21. Wong VW, Wong GL, Choi PC, et al. Disease Progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. *Gut* 2010; 59: 969-974.
22. Wong GL. Transient elastography: Kill two birds with one stone? *World J Hepatol* 2013; 5: 264-274.
23. Wong GL, Chan HL, Yu Z, Chan HY, Tse CH, Wong VW. Liver fibrosis progression is uncommon in patients with inactive chronic hepatitis B: a prospective cohort study with paired transient elastography examination. *J Gastroenterol Hepatol* 2013; 28: 1842-1848.
24. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. *Gastroenterology* 2012; 142: 1293-1302.e4.
25. Qi XT, Wan F, Lou Y, Ye B and Wu D. The mean platelet volume is a potential biomarker for cirrhosis in chronic hepatitis B virus infected patients. *Hepatogastroenterology* 2014; 61: 456-459.
26. Hu Y, Lou Y, Chen Y, Mao W. Evaluation of mean platelet volume in patients with hepatitis B virus infection. *Int J Clin Exp Med* 2014; 7(11): 4207-4213.
27. Gîrleanu I, Trifan A, Cojocariu C, Dimache M, Sîngeap AM, Stoica O, Sfarti C, Stanciu C Platelet indices in patients with de novo portal vein thrombosis and liver cirrhosis. *Rev Med Chir.* 2013;117(3): 641-647.
28. Lisman T, Porte RJ. Pitfalls in assessing platelet activation status in patients with liver disease. *Liver Int* 2012; 32(6): 1027.

NEWS

IMPACT OF BACTERIAL CELLS AND SECRETOMES ON WOUND HEALING

According to Brothers KM *et al.*, bacterial cells and secretomes from *Serratia marcescens* (95%), *Pseudomonas aeruginosa* (71%) and *Staphylococcus aureus* (29%) strains inhibit human corneal epithelial cell migration *in vitro* and *ex vivo*, thus affecting the process of wound healing. Lipopolysaccharide (LPS) seems to be a key factor in this inhibitory mechanism, since LPS core biosynthetic genes are required to inhibit corneal epithelial cell migration. Purified LPS from *S. marcescens* inhibits epithelial cell migration *in vitro* and wound healing *ex vivo*, while after LPS depletion of *S. marcescens* secretomes, migration of epithelial cells was no longer inhibited, suggesting that *S. marcescens* LPS is sufficient for inhibition of epithelial wound healing (Brothers KM, Stella NA, Hunt KM, Romanowski EG, Liu X, Klarlund JK, et al. Putting on the brakes: Bacterial impediment of wound healing. *Sci Rep.* 2015;5:14003. doi: 10.1038/srep14003.)

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THE MALFUNCTIONS OF A NATIONAL TRANSPLANTATION SYSTEM: MULTI-LAYERED EXPLANATIONS FROM WITHIN

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THE MALFUNCTIONS OF A NATIONAL TRANSPLANTATION SYSTEM: MULTI-LAYERED EXPLANATIONS FROM WITHIN (Abstract): Romania has one of the lowest posthumous organ donation and transplantation rates in Europe. **Aim:** To explore the perspectives on the national transplantation system deficits shared by the physicians responsible for the identification and referral of potential posthumous organ donors. **Material and methods:** The study consists of semi-structured interviews with the key informants (ICU physicians, neurologists and neurosurgeons) in county hospitals in Moldavia. **Results:** The explanations for the current Romanian organ transplantation rate, as emerged from the participants' answers, are multi-layered. Overall, the national transplantation system is accused of being plagued with serious problems: it is underfunded, understaffed, its resources have been distributed unevenly among medical centers, the communication between its elements is disrupted, leaving the majority of the transplant professionals practically detached from its upper layer and affecting their motivation to perform their transplantation-related tasks. Furthermore, some of the participants point to the strong reluctance of the population to donate their deceased relatives' organs. **Conclusions:** The results suggest a set of possible multi-level interventions that could alleviate these problems. **Keywords:** TRANSPLANTATION, ORGAN SHORTAGE, TRANSPLANT SYSTEM, HEALTH PROFESSIONALS, POPULATION, ROMANIA.

There is a universal shortage of cadaveric organ donors; while the number of patients waiting for transplants continues to increase, organ donation rates remain low. These rates vary widely between countries, Romania ranking near the bottom of the European hierarchy, with 3.6 deceased donors per million of the population in 2009 (1). Only 31% of the Romanians would agree to donate their organs after

death and 34% would consent to donate organs of a deceased family member, far below the European means of 55% and 53% respectively (2).

An important factor influencing the posthumous organ donation rates is the refusal of the bereaved families to give consent for donation and the public's reluctance to donate (3). The attitude of the health professionals towards organ dona-

tion and transplantation, as well as their knowledge on these issues, also influence the decision to donate of the family members (4, 5). Their influence is not limited to the conversation with the family members, but it extends to their own involvement in terms of identification and referral of potential organ donors (6, 7).

In addition, the characteristics of the national transplantation system influence the work of the health professionals involved (8, 9) and, consequently, the number of donated organs. Among others, the technological level of the available medical facilities, the adequacy of personnel training or the degree of articulation between the various levels of the medical system can hinder or stimulate the efficiency of the transplantation system.

The aim of our study was to explore the perspectives of the physicians involved in the identification of potential organ donors on the factors influencing the functioning of the Romanian transplantation system and on the available interventions that could limit the negative influences.

MATERIAL AND METHODS

We used a qualitative methodology based on semi-structured interviews with open-ended questions. The participants were physicians of one of the three specializations involved in the diagnosis of brain death (neurology, neurosurgery and ICU) working in hospitals from the Moldavian counties, and responsible for the identification and referral of potential brain death donors. 24 physicians were contacted; 17 agreed to participate (10 men, 7 women): 6 neurologists, 6 neurosurgeons and 5 ICU specialists. The participants' age ranged from 41 to 57 and their work experience from 3 to 19 years. The interviews lasted about 50

minutes each and were all recorded.

The study was approved by the Research Ethics Commission of the University of Medicine and Pharmacy "Grigore T. Popa"- Iași. All participants were informed about the aim and procedures of the study and freely consented to be enrolled.

The interviews were transcribed verbatim, and analyzed in a grounded theory approach. The interviews were coded using the county name and the medical specialty abbreviation (N- Neurology; NS- Neurosurgery; ICU- Intensive Care Unit). In order to access and explore the essential meanings of the answers, the constant comparative analytic procedure was employed, by first reducing them to concepts through open coding. Then, we explored the associations between the various categories in different parts of the textual data. Each hypothesis that emerged across this coding process was compared to the new statements in participants' answers, thus assessing its validity. When the meanings of the new statements differed from the previously established relevant hypotheses, the latter were reformulated in order to accommodate them. When the differences were too large, indicating the existence of distinct perspectives on the same issue, the new meanings were theoretically condensed into a new partial hypothesis.

RESULTS AND DISCUSSION

The inner problems of the Romanian transplant system

The participants pointed out several categories of inner problems, distributed on several layers, from the individual one, the human resources employed in the system, to more general, organizational issues. On the first layer, some of the respondents identified the health professionals' low

motivation. On one hand, their involvement in the transplant- related interventions is an additional task, interfering with the already busy daily work schedule:

"It is an extra activity and the not so numerous employees already have to cope with a busy schedule. It is natural for interest in such activity, that we consider collateral, to be reduced" (NS1).

Therefore, the level of effort invested in the organ transplant- related tasks is low:

"I think it is also about our lack of involvement" (NS2).

A possible solution to this motivational issue is, for some participants, the financial retribution of their involvement in the transplant- related tasks.

"Those in charge of transplants should be paid more, no doubt about it!" (ICU5)

On the other hand, the low external rewards of the physicians could be compensated by their internal motivation, grounded in the life-giving character of transplantation. The satisfaction stemming from saving the life of the organ recipient can help them rationalize the efforts they had invested in the lost cause of saving the life of the deceased donor:

"We often struggle for patients who we know to have little chance, and we see no solution, but maybe it is then we get a new one, a new meaning...of all the work invested. Despite the end of that particular life, a new one might continue" (ICU2).

This type of approach involves the health professional's concern for the welfare of the potential organ recipient. Altruism appears to be an important factor for the health professionals' approach on organ donation (10). However although altruism should be at the heart of the medical profession, its current practical relevance is diminished. This issue would require edu-

cational interventions directed to physicians, such as development of campaigns aimed at stimulating their altruistic tendencies as motivators for their involvement in the organ transplant- related tasks:

"I say 'education' because we should get involved in this field of activity selflessly, as a result of our belief. In the long run, this is our purpose in medicine, or at least it should be" (ICU1).

Beyond the motivational issues, some physicians point to a systemic dysfunction in the informational circuit between the levels of the Romanian transplant system. Its main consequence is the informational deprivation of the physicians, not only in terms of their scientific update on the relevant medical issues, but also concerning the basic statistics of the outputs of this system:

"Not even us, the doctors, have knowledge of the number of kidney transplants performed in Romania so far, as well as their consequences" (N2).

The limited personal experience of many physicians with organ transplantation- related procedures can influence their attitude towards organ donation and gradually instigate further reticence among this first and essential layer of the Romanian transplant system. Other studies reveal that an important variable in this respect is health professionals' amount of personal experience with managing and referring brain dead patients (11). On the other hand, even when potential donors are identified in these "lower level hospitals", the lack of feedback on the successful transplant procedures that are performed elsewhere further induces a sense of futility regarding their efforts. Providing follow-up information on organ recipients could prove an effective intervention in order to raise the

level of support for and involvement in transplantation- related tasks among these health professionals (12).

An explanation for the scarcity of the information transmitted to the lower levels of the system is the traditional, and still ongoing, status of transplant interventions as high-end procedures, reserved to a select group of professionals:

"The specialized literature has not presented this side of the transplant as a real treatment possibility, but more like some sort of performance...reserved to a limited number of doctors, having experience, conditions and so on...even now this topic is still viewed as high performance or excellence in medical practice" (N3).

At present, this situation is perpetuated by the insufficient funding only covering the organ transplant- related expenses of the large medical centers:

"Large centers alone have this possibility. Funds were granted and still just the first layer benefited from technology, money and specialists" (ICU1).

Another consequence of this lack of articulation of the transplant system is the deficient communication between its various levels on current relevant issues:

"I think the most important problem is the lack of communication between ICU and the donation team" (ICU4).

Given the fast-paced nature of the set of procedures required by organ retrieval, these communication issues hinder their success, even causing failures in potential donation cases:

"There was a case here in which everyone stuttered: who's responsible for transplant, who's the one doing this, who's the one doing that... when things were finally clear, it was too late" (NS 1).

The lack of adequate funding is a seri-

ous issue, invoked by all our respondents. It affects the performance of the transplant system in two ways. First, it leads to dramatic shortage in the necessary technical equipment, limiting, in some cases, the available transplant- related procedures:

"There is also a deficit concerning the county hospitals equipment, necessary for brain death diagnosis and for maintaining artificial ventilation. They are very scarce in ICU, where the priority is set on the patients with a higher chance of survival." (NS6).

Second, the insufficient funding creates personnel insufficiency, limiting the tasks that the medical staff can perform and, consequently, their involvement in the transplant- related procedures. Furthermore, these insufficiencies raise ethical challenges regarding organ transplant-related procedures, since they require the exclusive investment of a considerable part of these scarce resources, affecting the medical care offered to the other patients:

"If I have two nurses on my shift, one should see to the special care of the donor, which is highly unlikely, since I wouldn't know what to do with the other severe patients in the department. This would not be fair to the other patients." (ICU5)

Subsequently, the cost – benefit analysis of the current situation questions the overall utility of organ transplantation, especially in the hospitals that do not perform actual transplant interventions, but should merely identify potential donors.

Overall, the current under-financing of the medical system as a whole seriously affects the rationality of the transplantation sub-system. The lack of involvement of hospital professionals in the transplant-related tasks appears as a way of defending the functionality of the rest of the system,

focused on providing medical care to the sick, by not deploying it of the already scarce resources:

"If you don't have the money, you do not perform transplantations. The transplant procedures become possible when the system is fit for it. If you don't even have the foundation, you can't perform transplantations, it's as simple as that" (N2)

Nevertheless, in line with the issue of the uneven distribution of resources, some participants remarked the paradoxical character of the Romanian transplant system, mirroring the entire medical system. This paradox stems from the massive differences not only in funding, but also in specialized education and experience between the two levels of the system: the "closed circle" of the first-layer medical centers and the rest of the hospitals:

"[...] again, this is a reflection of the Romanian medical system, with very competent peaks and the contrasting rest of the medical mass, struggling for survival, due to the lack of personnel, equipment, funds and so on." (N3).

In order to alleviate these structural problems, the Romanian physicians mostly prescribe top-down interventions: higher financial investments at all the levels of the transplant structure and the increased democratization of the system. Generally, the experience of other countries (9) shows that organizational improvements can increase the efficiency of the transplant system and, ultimately, the organ transplantation rate.

The attitudes of the general population toward organ donation

The reluctance of the general population to consent to organ donation from their deceased next of kin is frequently invoked as a major cause of the shortage of availa-

ble organs for transplantation in many countries (13). Our participants pinpoint several factors contributing to the public's reluctance that have been identified by other investigations as predictors of people's willingness to donate. An important such factor is the lack of information on the topics of brain death and organ transplantation (14), which causes difficulties in explaining the diagnosis of brain death to the bereaved families (15):

"The public's lack of knowledge concerning transplantation is a frequent difficulty in discussing donation with the potential donors' families." (NS5)

The general lack of information is attributed by our respondents to the low involvement of the media in popularizing this topic:

"No kind of lobby for transplants, no mass media popularization, neither scientific, nor in terms of popular medicine have been made." (N2)

Moreover, the rare media reports of transplantation cases led to the perception of this procedure as an exceptional and unavailable medical option:

"People perceive this kind of procedure simply as an accidental performance." (N6)

Thus, a possible way to change people's attitudes toward organ donation is the more frequent media coverage of transplant interventions and organ donation. More extensive media coverage of this topic would alleviate the current "eccentricity" of organ transplantation in the public perception, which, in its turn, would positively influence people's attitudes. This idea is supported by previous results (16) showing that social advertisement of organ donation increases the occurrences of the organ donation topic in the discussions among fami-

ly members.

Another factor of the frequent reluctance of the Romanians to donate the organs of their next of kin, identified by some of the participants, is their low levels of altruism:

"My kin died, let others die, too! This is simply selfish and absurd!" (N1).

While altruism has long been recognized as an important internal motivator in these circumstances (17), it is usually conceived as a global orientation towards other people in general, close to the notion of 'humanitarian impulses' (18). However, the psychological deficit criticized by the health professionals in our sample mainly concerns the Romanians' "lack of civic sense" (N2), their lack of interest in the welfare of their peers. This perspective moves the reference point in the definition of altruism from the global level to the strictly national one and suggests that deeper issues in the area of the Romanians' collective identity could function as deterrents of the public's willingness to donate. Some of the participants suggested that media should take on a third mission, besides informing and raising awareness of organ donation, that of stimulating people's general altruism.

"Those more likely to donate have certain pieces of information and an open mind" (ICU2).

Finally, another factor identified by the participants as generating the high organ donation refusal rates in Romanian is the low level of trust that people have in the medical system as a whole. As a result, the relationships between the health professionals and their patients are frequently impaired by the latter's suspicions regarding some hidden personal interests of the former. Consequently, the requests for the

donation of their deceased relative's organs are met with reluctance:

"The patient coming to the hospital already feels that everybody is a thief and wishes him no good, and those who trust what we know we must do are fewer each day." (NS5)

Moreover, the physicians working in the smaller hospitals have to deal with yet another type of suspicion, concerning their professional skills. Due to their mistrust in the medical competence of the hospital representatives, many of the relatives of brain dead patients withhold their consent, refusing the disconnection of the artificial life-support systems:

"People don't really trust us around here, at least not enough to consent to disconnect their kin from the life support system." (NS6)

Medical mistrust has been confirmed by previous studies as a significant predictor of attitudes towards organ donation (19). Due to its ubiquitous character highlighted by the physicians confronting it, our results suggest that it could dramatically influence the Romanians' stances on this topic.

CONCLUSIONS

Our study shows that the Romanian transplantation system is plagued with serious problems. It is underfinanced, understaffed, the resources being distributed unevenly among medical centers. The communication between its elements is disrupted, leaving the majority of the physicians practically detached from its upper layer, of those performing actual transplant procedures. Consequently, these physicians' motivation to involve in the tasks that are reserved for them by this undemocratic state of affairs is affected. At the same time, a clear set of possible interven-

tions at various levels can be inferred. First, organizational changes are mandatory, not only larger financial investments, but also stronger articulation of the various levels of the transplantation system. The latter would mainly entail increasing the communication between its two current levels- the medical centers performing transplant surgeries and those responsible only for the identification and referral of potential donors- as well as narrowing of the gap between them in terms of information, resources and, ultimately, surgical procedures performed. Second, media campaigns are called for in order to fill the public's massive knowledge gaps on the

organ donation and transplantation and to stimulate people's altruistic perspectives on them. In addition the transplantation system could also benefit from campaigns addressing the health professionals' intrinsic motivations to involve in transplant-related tasks.

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REFERENCES

1. Council of Europe. 2012. International figures on donation and transplantation- 2011. *Newsletter Transplant* 2012; 15(1): 32- 4. Available at: <http://www.ont.es>.
2. European Commission. Europeans and organ donation. *Eurobarometer 2009*. Available at: <http://ec.europa.eu>.
3. Garrison RN, Bentley FR, Raque GH. There is an answer to the shortage of organ donors. *Surg Gynecol Obstet* 1991; 173:391-396.
4. Duke J, Murphy B, Bell A. Nurses' attitudes toward organ donation: an Australian perspective. *Dimens Crit Care Nurs* 1998; 17:264–270.
5. Flodén A, Kelvered M, Frid I, Backman L. Causes why organ donation was not carried out despite the deceased being positive to donation. *Transplant P* 2006; 38:2619- 2621.
6. Maloney R, Altmaier EM. Caring for bereaved families: Self-efficacy in the donation request process. *J Clin Psychol Med Settings* 2003; 10:251–258.
7. Druguş D, Leaşu F, Sechel G, Repanovici A, Azoică D, Rogozea L. Assessment of personal qualities of healthcare professionals – determining factor for the improvement of healthcare services in the Romanian health system. *Rev Med Chir* 2015; 119(3): 843-849.
8. Sadala ML, Lorençon M, Cercal M, Schelp A. Caring for organ donors: The intensive care unit physicians' view. *Heart Lung* 2006; 35:190-197.
9. Matesanz R, Miranda B. *Organ donation for transplantation. The Spanish model*. Madrid: Grupo Aula Medica, 1996.
10. Ríos A, López-Navas A, Ayala-García MA, et al. Multivariate Analysis to Determine the Factors Affecting the Attitudes Toward Organ Donation of Healthcare Assistants in Spanish and Mexican Healthcare Centers. *Transplant P* 2012; 44:1479-1481.
11. Jeon KO, Kim BN, Kim HS, Byeon NI, Hong JJ, Bae SH, Son SY. A study on knowledge and attitude toward brain death and organ retrieval among health care professionals in Korea. *Transplant P* 2012; 44:859-861.

12. Kiberd MC, Kiberd BA. Nursing attitudes towards organ donation, procurement, and transplantation. *Heart Lung* 1992; 21:106-111.
13. Sheery E, Conrad SL, Brigham LE, et al. Estimating the number of potential organ donors in the United States. *New Engl J Med* 2003; 349:667-674.
14. Morgan SE, Miller J, Arasaratnam LA. Signing cards, saving lives: An evaluation of the Worksite Organ Donation Promotion Project. *Commun Monogr* 2003; 69:253-273.
15. Chernenko SM, Jensen L, Newburn-Cook C, Bigam DL. Organ donation and transplantation: a survey of critical care health professionals in non-transplant hospitals. *Progress in Transplantation* 2005; 15: 69-77.
16. Prottas JM. Encouraging Altruism: Public Attitudes and the Marketing of Organ Donation. *Milbank Q* 1983; 61:278-306.
17. Kopfman JE, Smith SW. Understanding the audiences of a health communication campaign: A discriminant analysis of potential organ donors based on intent to donate. *J Appl Commun Res* 1996; 24:22-49.
18. Cleveland S. Personality characteristics, body image and social attitudes of organ transplant donors versus non-donors. *Psychosom Med* 1995; 37: 317-319.
19. Sanner M. Attitudes toward organ donation and transplantation. *Soc Sci Med* 1994; 38:1141-1152.

NEWS

PROSPECTIVE ANALYSIS OF 55 CASES OF TUBERCULOSIS MENINGITIS IN NORTH INDIA

Tuberculosis meningitis (TBM) is a serious public health problem in developing countries as it leads to significant mortality and residual neurological sequelae. Despite being an endemic country for TB, data regarding clinical, radiological and laboratory (biochemical and microbiological) parameters and final outcome of adult TBM patients is sparse in India. Analysis of such variables in various countries has shown association of various factors with the prognosis of the disease like age, stage of disease, level of consciousness, presence of extraneural TB, isolation of *Mycobacterium tuberculosis* from CSF, biochemical studies and hydrocephalus. The availability of such data in a high developing country may help in prediction of the prognosis of such patients and help in early intervention of preventive measures to improve the subsequent outcome of the patients. In conclusion, TBM is a serious extrapulmonary form of TB and should arise suspicion in mind of clinician based on clinical, laboratory and radiologic results. The present work was planned to frame a model for prediction of mortality in TBM cases by including variables of age >40 years, past history of tuberculosis (TB), presence of basal exudates and hydrocephalus (Kaur H, Sharma K, Modi M, et al. Prospective Analysis of 55 Cases of Tuberculosis Meningitis in North India. *Journal of Clinical and Diagnostic Research*. 2015, Vol-9(1): 15-19).

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CERVICAL SPINE LESIONS IN RHEUMATOID ARTHRITIS PATIENTS

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CERVICAL SPINE LESIONS IN RHEUMATOID ARTHRITIS PATIENTS (Abstract): Aim of the study: to gather clinical and laboratory data on rheumatoid arthritis patients with cervical spine damage (incidence and prevalence, correlation between duration of disease and the time of lesion onset, to assess signs and symptoms and the role of laboratory investigations). The spine is an axial organ with an important role in support and resistance. It is a pillar with a very complex morphological and functional structure. The vertebral column is crossed by many kinematic chains. The main problem of the cervical spine caused by rheumatoid arthritis is cervical instability which describes all cervical lesions that can lead to neurovascular damage or major disturbance of pain generating statics at movement. The evolving disease shows chronic inflammation of the synovium, which is a self-maintained process and an immunologically induced phenomenon. The chronic inflammation of the synovium forms granulation tissue that invades peripheral joints towards the center and causes ligament cartilage and bone damage. **Material and methods:** The present paper investigated cervical spine lesions in 107 rheumatoid arthritis patients who were admitted to the 1st Rheumatology Clinic of Iasi Rehabilitation Hospital between January 2013 and December 2014. Our study focused on assessing signs and symptoms seen in spine affected by rheumatic disease. **Results and Discussions:** the disease causes destructive lesions due to granulomatous infiltration of rachidian structures and medullary sheaths. These lesions lead to damaged discs and instability that produces subluxations and dislocations. The suboccipital region is most affected; in other regions of the spine, high lesions of C4-C5 prevail, where osteolysis damage of spinal apophyses are found. In atlas and axis joints, rheumatoid arthritis causes the inflammation of bursa, synovium and joint capsule and leads to synovial pannus formation. This causes the destruction of cartilage and subchondral bone. Atlantoaxial dislocation is caused by erosive synovitis of atlanto-epistrophic joint, atlanto-odontoid joint and serous bursitis separating the odontoid process from the transverse ligament. **Conclusions:** The dominant symptom of cervical spine damage was pain associated with stiffness and limited joint mobility, muscle stiffness, poor posture. **Keywords:** CERVICAL INSTABILITY, DISLOCATION, DESTRUCTIVE EROSIONS, RADIOLOGICAL CRITERIA.

Cervical spine lesions in rheumatoid arthritis (RA) patients lead to increased physical pain, with local radicular bone marrow involvement that aggravates the functional

deficit, worsening the vital prognosis. The impairment of the cervical spine can be explained only partly by its morphological and dynamic characteristics (1).

During the evolutive process, the chronic inflammation of the synovial membrane is a self-maintained, immunologically induced process that leads to the formation of granulation tissue invading peripheral joints towards the center and causing cartilage and bone ligament lesions (2).

Atlanto-axial dislocation occurs at the C1-C2 segment (anterior more than 3 mm between the arch of C1 and the C2 odontoid, with synovial proliferation in the C2 odontoid joint and the anterior arch of C1 that can lead to rupture of the transverse ligament and alar ligaments) (3).

Other lesions of the cervical segment caused by rheumatoid arthritis include: vertical axial subluxation = collapse of the lateral joints between C1 and C2; lateral atlantoaxial subluxation with pain in the frontal and suboccipital region and anterior inclination of the head posture; posterior atlanto-axial subluxation; C2 and C3 are the most commonly affected, while 10 to 20 percent of C3 and C4 are affected. The therapeutic intervention depends on the moment of survey or on the presence or absence of neurological signs (4).

Destructive lesions found in the rest of the cervical spine affect mainly the C3-C4 and C4-C5 segments, the lower cervical spine being less affected (5).

The present study focused on monitoring clinical and laboratory data of cervical spine lesions in rheumatoid arthritis patients.

Aims of the study: The evaluation of the incidence and prevalence of cervical spine damage in rheumatoid arthritis cases hospitalized in the 1st Rheumatology Clinic, Iasi Rehabilitation Hospital, the correlation between the duration of the disease and the onset of cervical spine lesions in

rheumatoid arthritis patients, the evaluation of signs and symptoms that occur in the rheumatoid damage of the cervical spine and the role of laboratory investigations in the diagnosis of vertebral instability (standard radiography, CT examination, MRI examination).

MATERIAL AND METHODS

The study included a group of 107 RA patients who were admitted to the 1st Rheumatology Clinic, Iasi Rehabilitation Hospital between January 2013 and December 2014.

The inclusion criteria were the 1987 ACR classification criteria for RA and the results of the radiological examination (2).

Exclusion criteria included RA patients who did not show clinical or radiological damage to the cervical spine and RA patients with only degenerative changes. The demographic characteristics in the group of patients were initially evaluated through descriptive statistical methods; statistical correlations between clinical and laboratory parameters (e.g. Pearson correlation) were subsequently performed.

T-student and chi-square tests were used for the statistical evaluation of data. They were clinically significant when p is less than or equal to 0.05. The database was systemized with Excel (Microsoft Office), and the statistical analysis used the statistical programs MedCalc and Epi Info 2000.

RESULTS

RA incidence and prevalence are reported differently according to the diagnostic criteria used in population studies (table I).

Out of the total number of 20 (18.69%) RA patients with cervical spine involvement admitted during the study period, 13

(12.14%) were women and 7 (6.54%) were men. A predominant involvement of female patients was noted, showing a regular sex distribution of RA patients and cases with

cervical lesions in the sense that female cases are about three times more frequent than male cases. Female gender is a factor of poor prognosis of the disease.

TABLE I
Incidence and prevalence in the study group

Year	2013	2014	Total
Total number of RA patients	46	61	107
RA with cervical damage - new cases	6	9	15
RA with cervical damage - reappraisal	2	3	5
Incidence of cervical damage in RA patients	13.33%	20.00%	33.33%
Prevalence of cervical damage in RA patients	17.39%	14.75%	32.14%

54.4% of the study group patients were from urban areas, possibly explained by the higher medical visit rate of the urban group. The values related to the duration

(in years) of RA were dispersed, ranging from 0 to 35 years of disease, most patients (64.5%) being in the group with a duration of less than 5 years (table II).

TABLE II
Distribution of the study group according to the duration of the disease

Years	< 5	5-10	10-15	15-20	>20
Patients in the study group (%)	61.31	14.50	5.60	3.40	4.40

The study group patients were aged over 45 years and cervical lesions were present in patients with a duration of the disease of at least 10 years in cases with onset before the age of 65 and occurred early in patients with senile RA. Damage to the cervical spine was higher in elderly patients with a longer duration of the disease and especially in the more severe types of disease.

Specific symptoms occurred gradually in most patients from a period of several months to several years. The dominant symptom was joint pain exacerbated at night associated with prolonged morning stiffness and swelling of various joint areas that have led to functional impairment, the higher, the more regions were affected by arthritis. Several clinical onset forms were observed, i.e. monoarticular, oligoarticular,

polyarticular. Other onset forms were extraarticular manifestations such as muscle pain, tendonitis or ductal syndromes (especially carpal tunnel syndrome). The polyarticular type of disease onset with rapid development of deformities and joint ankyloses was mostly seen in patients with cervical damage.

TABLE III
Distribution of general signs
in RA patients

General signs	%
Asthenia, fatigue	97
Loss of appetite	45
Transient arthralgia	91
Raynaud's syndrome, acrocyanosis	49
Weight loss	12
Irritability	83
Depression, insomnia	85

Cervical spine lesions in rheumatoid arthritis patients

The main clinical manifestations were pain, swelling, morning stiffness and functional discomfort (table III).

Inflammatory cervicalgia and cervical stiffness was present in all study group patients studied, but the elderly with senile RA, aged 65 years, cervicalgia showed also degenerative changes. Signs suggesting the localization of the rheumatoid process at the cervical level in the study group were found in varying proportions (tab. IV).

The dominant element was cervical and cervical-cephalic pain associated with stiffness of the extensor, trapezius or sternocleidomastoid muscle. Almost half of the patients showed a poor posture of the cephalic extremity with slight ante flexion.

16% of patients had severe neurological damage caused by cervical changes, while the altered sensitivity seen in the dermatomes of the cervical plexus roots was frequent.

TABLE IV
Symptoms of cervical damage in RA patients

Clinical signs of cervical damage in rheumatoid arthritis	%
Scalp tenderness	66%
Tenderness of the spinous apophyses, cervical chain	95%
Altered sensitivity of the cervical region and upper limbs	45%
Muscle tenderness	74%
Muscle weakness in regional muscles and upper limb end	54%
Fasciculations	1%
Transient hearing loss	3%
Sensory ataxia	1%
Sensitivity of the carotid sinus	9%

The study of global and segmental mobility of the cervical spine showed a severe limitation of movements in the upper region in 37% of the cases and an overall mobility limitation in 41% of the patients.

TABLE V
Extraarticular manifestations in RA study group patients

Extraarticular manifestation	%
Subcutaneous nodules	39.7
Tenosynovitis	31.0
Myopathy	36.3
Cardiomyopathy	6.1
Rheumatoid lung	24.3
Pleurisy	0.93
Pulmonary fibrosis	7.5

In patients with severe cervical damage, extraarticular manifestations included

changes in the skin, subcutaneous tissue and juxtaarticular tissues, respiratory and cardiovascular systems (table V).

Skin manifestations – 39.7% subcutaneous nodules were seen in patients with cervical lesions. This was correlated with a relatively long duration of the disease and with severe forms of the disease. They were associated with seropositive forms of the disease and with other systemic manifestations such as rheumatoid lung.

Nonspecific cutaneous manifestations (pale skin, hypopigmentation, dull brittle nails, hyperpigmentations) were also seen. Some of them express the inflammatory anemia caused by the long evolution of the disease in the selected patients.

Skeletal muscles and tendons - the study group patients often showed myalgia (in the shoulder girdle) and muscle weakness,

clinically expressed as muscle atrophy caused by inactivity, secondary to the joint pain of the disease. The anatomicopathological basis was represented by degenerative lesions of muscle fibers.

Respiratory manifestations - multinodular rheumatoid lung, radiographically shown, clinically manifested as dry cough, moderate dyspnea and intermittent hemoptysis were present in 14 (58.3%) patients. Pleurisy was observed in 1 (0.93%) patient; as the pleurisy was quantitatively reduced, it was reabsorbed following appropriate antibiotic and anti-inflammatory therapy.

Cardiovascular manifestations - difficult to assess, given that patients in the study group were over 45 years old and physiological changes of involution are closely intricately with pathological changes that cannot be properly classified. Chest pain, dyspnoea on exertion or tachycardia are difficult to interpret. Arterial hypertension was present in 63% of patients.

Digestive manifestations - clinically, 14 (35%) patients had hepatomegaly at baseline. Following administration of NSAIDs, 31% of patients had epigastralgia and heartburn. Gastric or gastroduodenal ulcer, radiographically shown, was present in 3 (15.00%) patients.

Neurologic manifestations - compressive neuropathies, such as carpal tunnel syndrome, were found in 9 (45%) patients and resulted from existing periarticular changes. The manifestations were more pronounced in the dominant limb. Damaged brachial plexus roots gave rise to radicular syndromes characterized by pain, paresthesia, hypoesthesia in the corresponding dermatome and the decrease of osteotendon reflexes. A significant percentage of patients showed this manifestation. These clinical changes were present mainly in patients with specific cervical

radiological changes, such as subaxial dislocation. Cervical-brachial neuralgia was seen in 7 (6.54%) patients.

Facial paralysis was observed in 2 (10%) patients, probably caused by the damage of the spinal nucleus of the trigeminal nerve, as patients had atlantoaxial dislocation. Several patients showed symptoms of dysphagia and dysphonia, which can be attributed to altered hypoglossal nerve (XII), vagus nerve (X) and glossopharyngeal nerve (IX). By using the Redlung-Johnell method, radiological changes such as vertical atlanto-axial dislocation were seen in these patients.

Renal manifestations. The research showed that 2 (10%) patients had a proteinuria of 1.5 g / 24 hours, one patient having a proteinuria of 1g / 24 hours. A high frequency of urinary tract infections was seen in women (38.6%) due to associated risk factors found in RA patients with decreased immune defense, hormonal changes, frequent hospitalizations and consumption of NSAIDs.

Hematologic changes - anemia was present in 17 (8.00%) patients (out of the total number of 20 patients). The most common cases showed normocytic and hypochromic anemia, as well as and hyposideremia; serum Fe values were low in some patients. The anemia seen in these patients can be attributed to the long evolution of the inflammatory disease and it is associated with iron absorption disorders, moderate decrease of erythropoiesis, hemolysis and blood loss.

3% of the study group patients had clinical manifestations of diplopia that might be caused by the damage of the abducens nerve (VI).

The following laboratory parameters and their correlations were followed in patients with cervical lesions caused by RA: biological inflammatory syndrome,

immunological changes - rheumatoid factors, standard radiological examination - flexion / extension; CT and MRI were used in patients with neurological symptoms.

Inflammatory syndrome - the assessment of ESR and CRP values, the blood count were useful for both diagnosis and the survey of the progression of the disease by determining their dynamics.

Anterior atlanto-axial dislocation was the most common form of cervical instability (65% of study group patients). Combined dislocations such as anterior atlanto-axial dislocation associated with the subaxial dislocation were far less numerous, 7 (35%) patients, as compared with 13 (65%) patients with anterior atlanto-axial dislocation. Other forms of dislocations were much less common.

The radiological examination has shown fractures of the odontoid in 1 patient; erosions of the interapophyseal joint in 4.2%; odontoid secondary osteosclerosis in 2.5%; the appearance of diffuse osteoporosis was associated with about 47% of the total number of patients. CT was performed in 4 patients and MRI in 3 patients.

DISCUSSIONS

Anterior atlanto-axial subluxation is the most frequent classical involvement of cervical spine in RA. The profile radiography of the cervical spine, C1-C2 - centered, shows a distance of more than 3 mm between the posterior edge of the anterior arch of the atlas and the anterior edge of the odontoid apophysis. The anterior subluxation of the atlas to the axis is possible due to the rupture or distension of the transverse and cruciform ligaments, following the chronic inflammation of the atlanto-axial joint (6, 2).

Subluxation causes the narrowing of the retro-odontoid part of the spinal canal and

the compromise of the neuraxis at the bulbo-medullary junction.

Clinically, the alteration of this joint can be completely asymptomatic, with a latent evolution, or may lead to severe cervical pain, high and posterior with inflammatory rhythm. Neurological complications are quite rare and include Lhermitte's sign or neurological signs of the upper limb: sensory disorders, movement disorders (3,4).

The literature reports also cases of sudden death (5), mostly following road accidents or during intubation for general anesthesia. Therefore, the arthritic condition must be systematically investigated before any surgical intervention (4,8).

Our findings showed also that the positive diagnosis is based on radiological examination (profile radiography of the cervical spine, C1-C2-centered, dynamic, flexion and extension) and on CT examination (a posterior detachment of the odontoid apophysis related to the anterior arch of the atlas is observed on the cross sections of the spine) similar to the reports of other authors (11,12).

The cervical spine damage in RA might involve also the middle and lower spine under the form of posterior interapophyseal arthritis. This could cause joint slipping or subluxations between vertebral bodies. These lesions are easily noticed on dynamic flexion / extension radiographs. These areas of the cervical spine are affected in 15% of the cases. The results concur with literature data already mentioned (13,14).

CONCLUSIONS

Our study showed that cervical spine lesions lead to increased physical pain, with local radicular bone marrow involvement that aggravates the functional deficit, worsening the vital prognosis.

The main disorder of the cervical spine

damaged by rheumatoid arthritis is cervical instability. The damage of the cervical segment caused by RA occurs late in the evolution of the disease, when destructive cartilage and bone lesions, axial deformities, extensive muscular atrophy and, in

some cases, even fibrous or bony ankylosis are present.

The dominant symptom of cervical spine damage was pain associated with limited joint mobility, muscle stiffness and poor posture.

REFERENCES

1. Narváez J, Narváez JA, Serrallonga M, *et al.* Subaxial cervical spine involvement in symptomatic rheumatoid arthritis patients: Comparison with cervical spondylosis, *Semin Arthritis Rheum*, 2015 Aug;45(1):9-17.
2. Joaquim AF, Appenzeller S. Cervical spine involvement in rheumatoid arthritis--a systematic review, *Autoimmun Rev*, 2014 Dec;13(12):1195-202.
3. Del Grande M, Del Grande F, Carrino J, *et al.* Cervical spine involvement early in the course of rheumatoid arthritis. *Semin Arthritis Rheum*. 2014 Jun;43(6):738-44.
4. Slater H, Briggs AM, Fary RE, *et al.* Upper cervical instability associated with rheumatoid arthritis: what to 'know' and what to 'do', *Man Ther*, 2013 Dec;18(6):615-9.
5. Kim DH, Hilibrand AS. Rheumatoid arthritis in the cervical spine, *J Am Acad Orthop Surg*, 2005;13:463-74
6. Laval-Servant, A., Anatomie fonctionnelle. Faculté de Médecine, Université Pierre et Marie Curie, Paris, 2007.
7. Newton, Kim; Claudius, Ilene. Neck. În: Marx, John A; Hockberger, Robert S; Walls, Ron M. *Rosen's Emergency Medicine*. Ediția a 8-a. Philadelphia, Saunders, 2014.
8. Moore, Keith L; Dalley, Arthur F; Agur, Anne MR. *Clinically Oriented Anatomy*. Ediția a 6-a, Philadelphia, Wolters Kluwer, 2010.
9. Alcalá JM, Douat D, Pinheiro DL, *et al.* Radiographic changes of cervical spine in rheumatoid arthritis. *Rev Bras Reumatol*. 2013 Sep-Oct;53(5):388-93.
10. Janteș B.M., Șuțeanu Șt., Afectarea scheletului axial în bolile inflamatorii reumatismale(I), *Rev.Reumatol*, 2003, 1-2(XI), 81-88
11. Boloșiu H.D., *Teme alese de reumatologie*, Ed.Medicală Universitară "Iuliu Hațieganu" Cluj – Napoca, 2003
12. Hochberg C.M., Silman A.J., Smolen J.S., Michael E., Weinblatt, Michael H Weisman.*Practical Rheumatology* in Les Barnley ed Neck pain. Mosby 2004, 13:140-151.
13. Rezuș Elena, *Reumatologie*, Ed. Gr. T. Popa, Iași, 2014.
14. Colebatch AN, Edwards CJ, Østergaard M, van der Heijde D, Balint PV,D'Agostino MA, *et al.* EULAR recommendations for the use of imaging of the joints in the clinical management of rheumatoid arthritis, *Ann Rheum Dis* 2013;72:804-14.
15. Casey ATH, Crockard HA. The cervical spine. In: Firestein GS,Panayi GS, Wollheim FA (eds). *Rheumatoid Arthritis*. 2.ed. London: Oxford University Press, 2006; p.475-84.
16. Kaupasi MJ, Barcelos A, da Silva JAP. Cervical complications of rheumatoid arthritis, *Ann Rheum Dis*, 2005;64:355-8.
17. Wasserman BR, Moskvicich R, Razi AE. Rheumatoid arthritis of cervical spine, *Bull NYU Hosp Joint Dis*, 2011;69:136-48.
18. Kaito T, Hosono N, Ohsima S, Ohwaki H, Takenaka S,Fujiwara S, *et al.* Effect of Biological Agents on Cervical Spine Lesions in Rheumatoid Arthritis, *Spine*, 2012 Apr 2.
19. Juan Marcelo Fernandez Alcalaa, Diogo Douatb Fábio Raimundo M dos Santosa, Marília B Silvaa, Thelma L Skarea,, Diogo Lago Pinheirob, Radiographic changes of the cervical spine in rheumatoid arthritis., *Rev Bras Reumatol*, 2013; 53(5) :388-393.

CORRELATIONS BETWEEN ARTERIAL HYPERTENSION INDUCED BY ANGIOTENSIN II AND SYSTEMIC INFLAMMATION IN RAT

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CORRELATIONS BETWEEN ARTERIAL HYPERTENSION INDUCED BY ANGIOTENSIN II AND SYSTEMIC INFLAMMATION IN RAT (Abstract): Angiotensin II has particularly shown to play a key role in the regulation of inflammatory processes in hypertension. **Aim:** The present study aims to correlate the angiotensin II – induced hypertension with systemic inflammation. **Material and methods:** We conducted an experimental study on Wistar male rats who received Ang II via subcutaneous miniosmotic pumps for 2 weeks. Rats were exposed to a 12h light /12h dark cycle. Sham rats were used as control. Systolic blood pressure measurements and a flow cytometric analysis of lymphocyte surface markers were performed. After 14 days, the animals were euthanized under anesthesia with xylazine/ketamine. **Results:** Systolic BP progressively and significantly increased in rats with Ang II chronic infusion. We observed a statistical significant difference ($p = 0.00001$), in terms of T lymphocytes percentage between control rats plasma and Ang II treated rats plasma, in 14 days. **Conclusions:** Angiotensin II is an important mediator of hypertension and directly promotes inflammation by noticeably increasing the quantity of T cells in kidney tissue. **Keywords:** ANGIOTENSIN II, HYPERTENSION, INFLAMMATION.

Hypertension is prevalent in 25% of the adult population and contributes to the development of coronary heart disease, stroke and chronic kidney disease (1). In 2003, the World Health Organization - International Society of Hypertension (WHO-ISH) provided a definition of hypertension as arterial pressure measurements of 140/90 mm Hg or greater (2). In only 5%-10% of patients, the causes of high blood pressure are identified. For the rest of cases the etiology remains unknown, and the hypertension is defined as “essential”.

Numerous studies showed that renin angiotensin aldosterone system (RAAS) contributes to cardiovascular and renal homeostasis by generating angiotensin peptides, mainly angiotensin II (Ang II). It has been shown that increased levels of Ang II cause hypertension, cardiovascular hypertrophy (1), increased vascular reactivity to different vasoconstrictor agents (1,3), inflammation (4) and endothelial dysfunction (5). Ang II causes a mild increase in blood pressure having as results T cell activation and inflammation, these factors having as result a

sustained hypertension (6). The present study intends to highlight some correlations between arterial hypertension induced by angiotensin II and systemic inflammation.

MATERIAL AND METHODS

Male Wistar rats (250–260 g) (n=9) received Ang II (300ng/kgc/min) in NaCl 0.9% via subcutaneous miniosmotic pumps, for 2 weeks as previously reported (7). Rats were exposed to a 12h light /12h dark cycle. Sham rats were used as controls (n=9). Care, use, and treatment of all animals in this study were in strict agreement with the guidelines of the animal care from the University of Medicine and Pharmacy "Grigore.T. Popa" Iasi. Basal systolic blood pressure (SBP) measurements were performed for every rat by tail-cuff plethysmography, twice/week at a basal period. After 14 days the animals were euthanized under anesthesia with xylazine/ketamine and we collected blood samples. Flow cytometric analysis was used to study lymphocyte surface markers. Comparisons within groups were performed using paired Student's t-test.

RESULTS

Systolic blood pressure. Before the treatments, systolic blood pressure was

comparable in the two groups (~120 mmHg). Though, systolic BP gradually and significantly increased in animals with Ang II chronic infusion (208±2 mmHg for Ang II versus 120±5mmHg for control group, in the 14th day).

Cellular viability in plasma. In the Ang II treated rats plasma we observed a statistical significant increasing number of PI-positive cells (non-viable) compared to cells derived from control rat plasma (fig. 1).

The cellular viability in plasma from control rats, respectively Ang II treated rats, is shown in fig. 2 and 3.

T and B lymphocytes into plasma. In figures 4 and 5 we revealed the presence of T and B lymphocytes in rat plasma (control lot vs. Ang II lot). We observed a statistical significant difference ($p = 0.00001$), in terms of T lymphocytes percentage between control rats plasma and Ang II treated rats plasma, for 14 days (fig. 6). As shown in figure 7, the percentage of B lymphocytes plasma, was statistically significant higher on rats treated with Ang II for 14 days ($p < < 0.05$) compared to control lot.

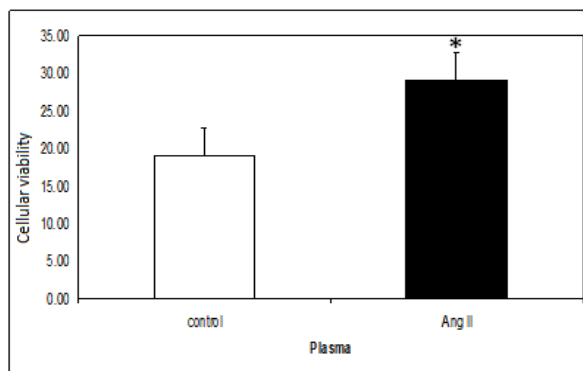


Fig. 1. Cellular viability percentage in rats plasma (control; Ang II) (* $p=0.002$)

Correlations between arterial hypertension induced by angiotensin II and systemic inflammation in rat

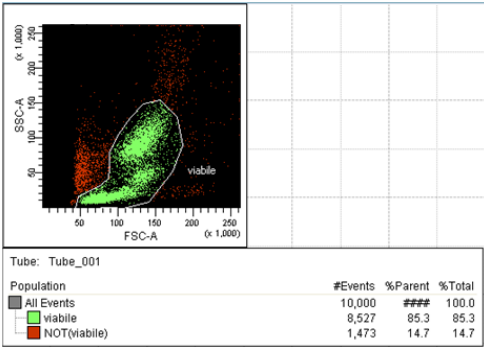


Fig. 2. Cellular viability in plasma (control lot)

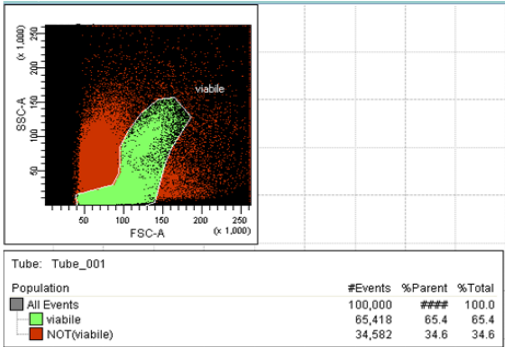


Fig. 3. Cellular viability in plasma (Ang II lot)

T and B lymphocytes into plasma. In figures 4 and 5 we revealed the presence of T and B lymphocytes in rat plasma (control lot vs. Ang II lot). We observed a statistical significant difference ($p = 0.00001$), in terms of T lymphocytes percentage between

control rats plasma and Ang II treated rats plasma, for 14 days (fig. 6). As shown in figure 7, the percentage of B lymphocytes plasma, was statistically significant higher on rats treated with Ang II for 14 days ($p < 0.05$) compared to control lot.

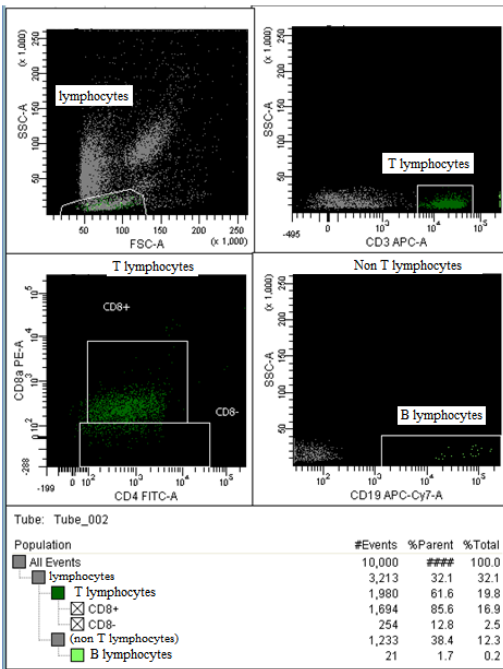


Fig. 4. T and B lymphocytes in plasma (control lot)

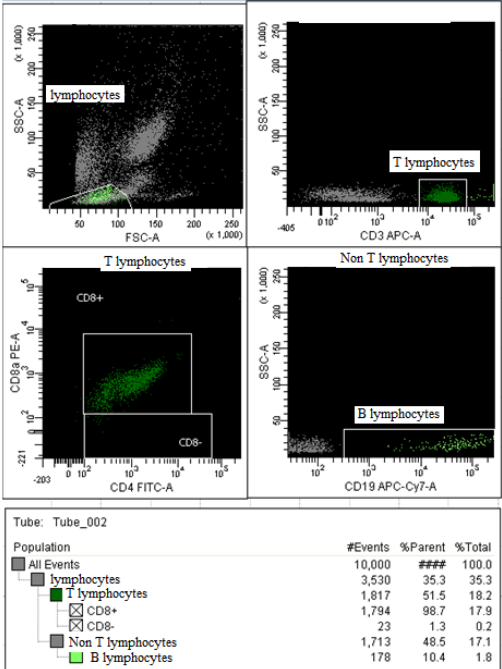


Fig. 5. T and B lymphocytes in plasma (Ang II lot)

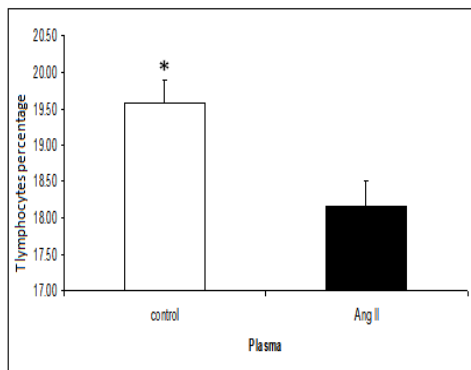


Fig. 6. Percentage of T lymphocytes in plasma (* $p = 0.00001$)

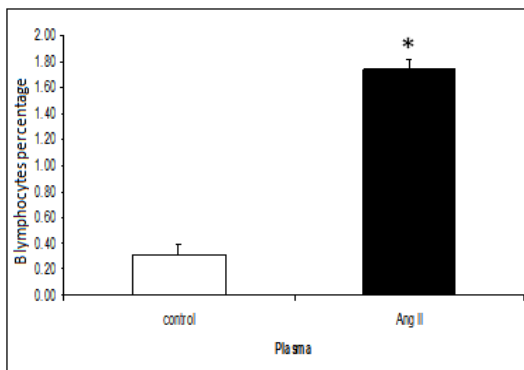


Fig. 7. Percentage of B lymphocytes in plasma (* $p < 0.05$)

DISCUSSION

Hypertension should be considered as a potential multifactorial inflammatory disease. Morphological abnormalities in the renal parenchyma and arteries seem to determine hypertension. Inflammatory processes might induce renal vasoconstriction, ischemia and injury that could sustain systemic hypertension. Arterial and tubulointerstitial inflammatory cells infiltration in response to renal damage might further increase renal and vascular alterations through the production of oxidants and other soluble inflammatory mediators (7,8). Increased levels of blood pressure may induce proinflammatory and pro-coagulant responses.

Up-regulation of local and systemic inflammatory mediators, such as components of the renin-angiotensin system (RAS), endothelial adhesion molecules, chemokines, cytokines and tissue factor (TF), was demonstrated in essential hypertension (9,10,11). The renin angiotensin aldosterone system (RAAS) contributes to cardiovascular and renal homeostasis by generating angiotensin peptides, mainly angiotensin II (Ang II). The effector of the RAAS, angiotensin II, was known mainly as a humoral

factor formed by the action of kidney-derived rate limiting enzyme *renin* (12).

Angiotensin II directly modulates cytokine release (13), endothelial adhesion molecule expression and the production of plasminogen activator inhibitor-1 (PAI-1) (14), thus favoring inflammatory vascular and renal damage (15, 16). These studies indicate that angiotensin II, in addition to its well-known cardiovascular effects, could also favor leukocyte recruitment within renal parenchyma in essential hypertension.

The flow cytometry results of the present study comparing control and Ang II treated animals noted that there is a statistically significant difference ($p = 0.00001$) in the percentage of T lymphocytes in plasma derived from control group rats compared to the percentage of T lymphocytes derived from the plasma of Ang II treated group for 14 days. Also, the percentage of B cells was significantly higher in plasma of rats that were treated with Ang II for 14 days ($p < 0.05$) compared to the percentage of B cells derived from the plasma of controls, confirming hypotheses from the literature regarding the involvement of angiotensin II in the development of inflammatory reactions.

CONCLUSIONS

Our paper reports that T cells play an essential role in the genesis of hypertension and support the involvement of inflammation in the occurrence of this disease. In our study, angiotensin II increased T cell markers activation. Angiotensin II markedly increased the number of T cells in plasma. Thus T cells might represent a novel therapeutic target for the treatment of high blood pressure.

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REFERENCES

1. Bucher BS, Tschumi S, Simonetti GD. Childhood's determinants for high blood pressure in adulthood. *Ther Umsch* 2012; 69: 295–298.
2. Whitworth JA; World Health Organization, International Society of Hypertension Writing Group. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens* 2003; 21:1983-1992.
3. Matrai M, Szekacs B, Mericli M, Nadasy GL, Szekeres M, Banhidy F, Bekesi G, Monos E, Várbíró S. Biomechanics and vasoreactivity of female intramural coronaries in angiotensin II induced hypertension. *Acta Physiol Hung* 2010; 97(1):31-40.
4. Androulakis ES, Tousoulis D, Papageorgiou N, Tsioufis C, Kallikazaros I, Stefanadis C. Essential hypertension: is there a role for inflammatory mechanisms? *Cardiol Rev* 2009; 17(5):216-221.
5. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension. Clinical implications and therapeutic possibilities. *Diabetes Care* 2008; 31(Suppl 2): S170–S180.
6. Marvar PJ, Thabet SR, Guzik TJ, Lob HE, McCann LA, Weyand C, et al. Central and peripheral mechanisms of T-lymphocyte activation and vascular inflammation produced by angiotensin II-induced hypertension. *Circ Res* 2010; 107: 263–270.
7. Touyz RM. Molecular and cellular mechanisms in vascular injury in hypertension: role of angiotensin II. *Curr Opin Nephrol Hypertens* 2005; 14(2):125-131.
8. Montecucco F, Pende A, Quercioli A, Mach F. Inflammation in the pathophysiology of essential hypertension. *J Nephrol* 2011; 24(01): 23- 34.
9. Stefanadi E, Tousoulis D, Androulakis ES, et al. Inflammatory markers in essential hypertension: potential clinical implications. *Curr Vasc Pharmacol*. 2010; 8(4):509-516.
10. Zhao Q, Ishibashi M, Hiasa K, Tan C, Takeshita A, Egashira K. Essential role of vascular endothelial growth factor in angiotensin II-induced vascular inflammation and remodeling. *Hypertension* 2004; 44: 264-270.
11. Chung NA, Beevers DG, Lip G. Effects of losartan versus hydrochlorothiazide on indices of endothelial damage/dysfunction, angiogenesis and tissue factor in essential hypertension. *Blood Press* 2004; 13: 183-189.
12. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol Rev* 2007; 59: 251–287.
13. Schieffer B, Luchtefeld M, Braun S, Hilfiker A, Hilfiker-Kleiner D, Drexler H. Role of NAD(P)H oxidase in angiotensin II-induced JAK/STAT signaling and cytokine induction. *Circ Res* 2000; 87:1195-1201.

14. Wojewódzka-Zeleznikowicz M, Stankiewicz A, Malinowska-Zaprzalka M, Chabielska E. The influence of tissue and plasma angiotensin converting enzyme inhibitors on haemostasis with respect to experimental and clinical investigations. *Kardiol Pol* 2005; 63(4 Suppl 2):S420-427.
15. Minela Aida Maranduca, Claudia Cristina Tarniceriu, Eugen Carasevici, Elena Cojocar. Interaction between angiotensin II, hypertension and inflammation in rat kidney, *Rev Med Chir* 2015, 119(3):791-97.
16. Haulica I, Bild W, Boisteanu D, Biosynthesis and physio-pharmacological actions of angiotensin peptides: 2. Physio-pharmacological properties, *Rev Med Chir* 2006, 110 920:384-90.

NEWS

RELATIONSHIP OF CAFFEINE WITH ADIPONECTIN AND BLOOD SUGAR LEVELS IN SUBJECTS WITH AND WITHOUT DIABETES

Insulin resistance or abnormal insulin secretion, are the characteristics of type2 diabetes mellitus (DM2), resulting in a diminished body glucose disposal. Those with chronic hyperglycemia, insulin resistance, or DM2 are at greater threat for its associated risk factors like hypertension, dyslipidemia, and cardiovascular disease. Genetic background and diet are considered to be one of the risk factors for developing type2 diabetes. Interestingly, among the several factors present in diet, coffee is considered as a potent dietary-component associated with reduced risk of diabetes and its complications. Most generally consumed beverage is coffee in the world, and its beneficial effects on health have been attracting substantial consideration. The common protective effect of coffee against diseases like cancer, cardiovascular diseases and type 2diabetes mellitus has been considered. Better glucose tolerance and a substantially lower risk of type 2 diabetes have been connected with high coffee consumption in diverse populations. The components responsible for the obvious beneficial effect of coffee on glucose metabolism remains blurred. Animal studies have shown that intake of the coffee components like chlorogenic acid, quinic acid and trigonelline, has enhanced glucose metabolism. Intervening metabolic studies on short term in humans have shown that caffeine can intensely lower insulin sensitivity. However, a regular high caffeine intake on long term has been linked with improved insulin sensitivity. An important secretory product of adipocytes is adiponectin, a marker and perhaps a mediator of metabolic and cardiovascular disease risk acts as a hormone with anti-inflammatory and insulin sensitizing properties. Further adiponectin levels are low in insulin-resistant subjects regardless of their obesity. A growing body of evidence has shown that high adiponectin levels confirm a protective effect against glucose intolerance budding in patients who are at high risk for diabetes. Further, it might be anticipated that the adiponectin level be affected with coffee consumption. In conclusion, the study shows the long term use of caffeine is more efficient on blood sugar and adiponectin levels, which needed in the prevention of complications in diabetic subjects (Bhaktha G, Nayak BS, Mayya S et al.Relationship of Caffeine with Adiponectin and Blood Sugar Levels in Subjects with and without Diabetes. *Journal of Clinical and Diagnostic Research*. 2015, Vol-9(1):1-3).

Doina Butcovan

SHORT TERM EVALUATION OF PERIMETRIC PROGRESSION IN PATIENTS WITH OPEN ANGLE GLAUCOMA AND DIABETES

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SHORT TERM EVALUATION ON VISUAL DECLINE IN PATIENTS WITH OPEN ANGLE GLAUCOMA AND DIABETES (Abstract). **Aim of study:** progression rate in patients with glaucoma and diabetes. **Material and method:** cohort prospective study in Ophthalmology Clinic - “Sf. Spiridon” University Hospital – Iași. We recruited patients with positive history for diabetes and of open angle glaucoma (OAG). The control group included subjects with OAG (74 eyes from 74 patients) and the study group (44 eyes, from 44 patients) included subjects with OAG and diabetes. At enrollment all patients had a complete ophthalmologic evaluation along with full metabolic status assessment. There were included only incipient and moderate forms of glaucoma, with mild or no diabetic retinopathy changes. Perimetric progression was assessed at 24 months with automated methods. **Results:** globally, from 118 investigated eyes, 56.40% cases had primary open angle glaucoma, 41.03% normal tension glaucoma and 2.36% pseudoexfoliative glaucoma. Glaucoma severity classification showed early defects (mean deviation < -6 db) in the study group of 77.27% vs. 83.78% in control group, whereas moderate defects (mean deviation > -6 db) were found in 22.63% in study group vs 16.21.0% in control group. Mean age of the patients was higher in absolute value in the open angle glaucoma group (64.31 +/- 1.66 years), vs diabetes + open angle glaucoma (62.69 +/- 1.8 years), with comparable visual acuities (0.91 +/- 0.15 vs 0.89 +/- 0.16), C/D ratios and other clinical parameters ($p > 0.05$). Mean baseline IOP in the study group was 18.18 +/- 3.55 mmHg vs. 17.08 +/- 2.4 mmHg in controls ($p > 0.05$). Analysis of visual field parameters at baseline showed a significant difference ($p = 0.048$) between groups in MD levels -3.63 +/- 3.35 db (control group) vs -4.40 +/- 5.78 db (study group), but no difference ($p > 0.05$) in PSD levels: 3.71 +/- 3.06 db (control group) vs 4.05 +/- 3.04 dB (study group). Perimetric progression was estimated at 24 months by Glaucoma Progression Analysis software (GPA – Humphrey Visual Field Analyzer II) using 6 reliable visual field (VF) exams. Progression rate was similar between groups - 0.19 ± 0.78 dB/year (OAG) vs. - 0.18 ± 0.05 dB/year (OAG+DM), $p > 0.05$ and no other risk factor could have been linked to an increased progression rate, except visual field parameters at final and baseline evaluation, in both groups. **Conclusions:** on short term, functional deterioration in open angle glaucoma patients having early or moderate stages, occurs similarly in the presence or absence of diabetes. **Keywords:** SHORT TERM, PERIMETRIC PROGRESSION, OPEN ANGLE GLAUCOMA, DIABETES.

Glaucoma represents an optic neuropathy that can lead to optic nerve irreversible

damage and blindness. Among all glaucoma types, primary open angle glaucoma is

the most common form and a leading cause of visual loss worldwide (1).

Individual evolution is highly variable, despite adequate treatment (2,3). In consequence, visual field changes and progression rates differ greatly (4,5).

Recent literature data mention the contribution of risk factors in glaucoma progression such as older age, decreased corneal thickness, pseudoexfoliation, low ocular perfusion pressure, disk hemorrhages, baseline visual field parameters or systemic diseases like diabetes, hypertension and cardiovascular events (3,6). Thus, the aim of our study was to assess if progression rate in patients with open angle glaucoma and diabetes is different on short term evaluation (24 months).

MATERIAL AND METHOD

Our study was designed as cohort prospective clinical trial and included a total of 118 eyes from 118 patients (73% males, 27% females), recruited in the Ophthalmology Clinic - "Sf. Spiridon" University Hospital - Iași, between October 2012-March 2014. The patients were divided in two groups as following: 44 eyes, from 44 patients in the study group (diabetes + open angle glaucoma) and the control group with 74 eyes from 74 patients with open angle glaucoma (primary open angle glaucoma, normal tension glaucoma and pseudoexfoliative glaucoma). At enrollment all patients had a complete ophthalmologic evaluation and metabolic status assessment. We included only incipient and moderate forms of glaucoma, with mild or no diabetic retinopathy changes.

The diagnosis of glaucoma was defined in the presence of open anterior chamber angle on gonioscopy, glaucomatous optic disc damage on clinical examination (focal

or diffuse neuroretinal rim thinning, localized notching, or nerve fiber layer defect) and corresponding visual field (VF) defects. Glaucoma severity was graded according to Hodapp criteria (7). Diabetes was confirmed by previous tests in our patients in the Diabetology Department. We selected only those cases with no/mild form of diabetic retinopathy.

At baseline we recorded clinical parameters that included: age, gender, best corrected visual acuity (BCVA) by ETDRS chart, intraocular pressure (IOP) by Goldmann tonometer, CCT by ultrasonic pachymeter (DGH-550, DGH Technology Inc., Exton, PA, USA) CD ratio (Volk 78D lens), number of topical medications, VF test parameters; at each follow up visit VA, IOP and VF tests were repeated. During the study the patients were followed each 3 months, when identical tests were performed. The last visit evaluated visual acuity, IOP, VF parameters. Majority of patients required topical therapy with 1-2-3 standard antiglaucoma medications. During monitoring, treatment was modified if the IOP was not efficiently controlled. Intermediary IOP was calculated by averaging all the IOPs taken during the 24 months follow up interval.

Exclusion criteria were related to significant lens opacities, other ophthalmic comorbidities, refractive errors >5D spherical, >3D cylinder. If both eyes were eligible, only one was chosen based on the worse mean deviation (MD) level at baseline

In Standard Achromatic Perimetry (24-2 SITA Standard SAP, Humphrey Field Analyzer II, Carl Zeiss Meditec Inc., Dublin, CA, USA) VF changes for glaucoma were defined if at least two of the three Anderson's criteria were fulfilled (three or more non-edged points in a cluster depressed to $P < 5\%$ and one of which depressed to

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$P < 1\%$, Glaucoma Hemifield Test outside normal limits and pattern standard deviation depressed to $P < 5\%$). Tests with fixation losses, false-positive or false-negative rates $> 20\%$ were excluded from the analysis. 6 reliable visual fields were analyzed from each patient and the first two tests served as baseline landmark; progression was evaluated by Glaucoma Progression Analysis software. The event-based progression analysis, Glaucoma Progression Analysis (GPA) software used statistical criteria designed for the Early Manifest Glaucoma Trial to identify progression of VF defects (10). When significant ($P < 0.05$) deterioration was evident on the pattern standard deviation (PSD) maps at the same three or more points on two consecutive follow-up tests, the GPA alarmed for 'possible progression'; if significant deterioration was seen at the same three or more points in three consecutive follow-up tests, GPA alarmed for 'likely progression'. When both the above criteria were not met, the software flagged as 'no progression detected'. For the purpose of this study, progression was quantitatively assessed by linear regression analysis of the mean deviation (MD) changes over time; slopes of progression (decibels/ year) based on threshold maps and its level of significance (p-values) were calculated.

During the study the patients were followed each 3 months, when identical baseline tests were performed.

A SPSS 18.0 statistical software (SPSS Inc. Chicago, IL, USA) was used to process the data. Descriptive analysis was used on demographics, follow up time MD, PSD and IOP. Progression rate (MD slope) was calculated by linear regression analysis of MD values over time and expressed in dB/year. Independent samples t tests were used

for comparisons of continuous variables between groups. Also when paired groups were compared (baseline and final parameters) we used Wilcoxon test. Spearman's correlation coefficients (r) were calculated to assess the relationship between age, BCVA, MD, PSD, IOP and MD slope. Statistical significance was defined at the $p < 0.05$ level.

RESULTS

From all the 118 eyes included in our study, 58.96% cases had primary open angle glaucoma (POAG), 41.03% cases had normal tension glaucoma (NTG) and 2.56% cases with pseudoexfoliative glaucoma (PXG), as shown in fig. 1. Sex ratio (F:M) was 1:2.9 (study group) and 1:1.5 (control group).

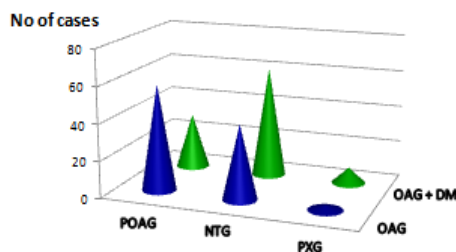


Fig. 1. Glaucoma type distribution in the two groups

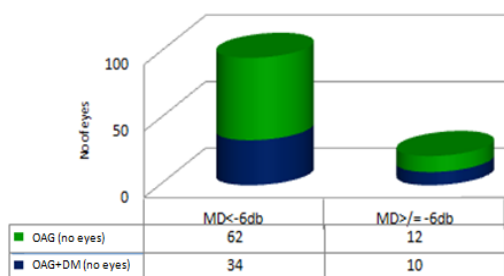


Fig. 2. Glaucoma severity classification (Hodapp criteria) in the 2 groups

Glaucoma severity classification showed early defects (MD < -6 db) in the study group of 77.27% vs. 83.78% in control group, whereas moderate defects (MD > -6 db) were found in 22.63% in the study group versus 16.21% in the control group, shown in fig. 2.

Baseline parameters and their evolution compared between the two groups were synthesized in table 1. The only statistical-

ly significant difference we found was related to the MD level at start, as the eyes with diabetes and OAG were more depressed in retinal sensitivity compared to the cases that had only OAG. All the other parameters were comparable and with no significant differences, meaning that we could compare homogenous groups, although the number of cases was not equal between them.

TABLE I
Parameters compared between groups at initial visit and during follow up

Baseline parameter	OAG+DM	OAG	T Student Test
Eyes (no)	44	74	nss
Age	62.69+/-1.8	64.31+/-1.66	nss-
Sex ratio (F:M)	1 : 2.9	1 : 1.5	nss
BCVA-ETRDS	0.91+/-0.15	0.89+/-0.16	nss
Sph. Eq	0.34+/-0.24	0.32+/-0.19	nss
CCT (µm)	538±35.9	528±62.8	nss
IOP (mmHg)	18.18±3.55	17.08±2.4	nss
MD (dB)	-4.40 ±5.78	-3.63+/-3.35	p=0.02
PSD (dB)	4.05±3.04	3.71±3.06	nss
No. of medications	1.43±0.90	1.62+/-1.13	nss
Follow up parameters			
VF exams	6	6	-
BCVA-ETRDS	0.85±0.21	0.83±0.21	nss
IOP (mmHg)	16.49±2.81	16.62±2.90	nss
MD (dB)	-5.64±6.89	-5.3±6.25	nss
PSD (dB)	4.38±3.49	4.22±3.49	nss
MD slope (dB/ year)	-0.19 ± 0.78	-0.18± 0.05	nss

nss – not statistically significant

In evolution at 24 months we observed in the study group (OAG and diabetes) a statistically significant change in IOP level

(18.18+/-3.55 mmHg vs. 16.49+/-2.81 mmHg, p=0.03) and in mean MD level (-4.40 ±5.78 vs. -5.64±6.89, p=0.04), aspect

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shown in fig. 3,4.

Rate of glaucoma progression (MD slope) at 24 months was similar and did not

differ statistically, but only in absolute value: -0.19 dB/year (OAG+DM) versus -0.18 dB/year (OAG), $p=0.55$ (t test).

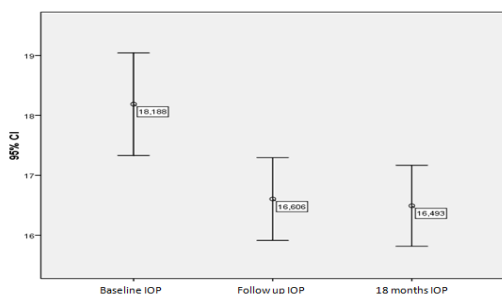


Fig. 3. IOP variation in the study group (OAG+DM): initial vs. follow up vs. final visit (24 months)

A more significant decrease in IOP level in the study group between baseline and after 24 months of follow up could be interpreted in the context of a more aggressive treatment applied initially to the patients with combined disease (OAG+DM) based on a hypothesis that all eyes are more prone to a more significant visual decay than the control group.

Spearman's coefficient detected significant correlations in the study group between MD slope and final/ initial MD levels ($p=0.000$), but also with baseline IOP ($p=0.032$). Intermediary IOP was correlated with final PSD ($p=0.04$), baseline IOP ($p=0.000$) and baseline MD ($p=0.016$). Both final and baseline visual field parameters were highly correlated, as expected at a $p=0.000$. Interestingly, MD slope in the study group was correlated ($p=0.043$) with the number of topical medications, but when age was controlled, the correlation became not significant any more.

In the control group we found the same type of correlations between MD slope and

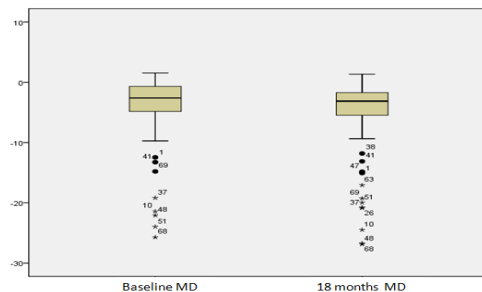


Fig. 4. MD mean values – baseline vs. 24 months in the study group (OAG+DM)

visual field parameters. Thus MD slope was correlated both with these parameters at baseline MD ($p=0.039$) PSD ($p=0.011$) and final MD level ($p=0.011$).

DISCUSSIONS

Progression rate and risk factors are among the most important aspects in glaucoma care because of their impact upon visual decay (8, 9, 10).

We found that visual field progression rate (MD slope) in both groups was dependent and correlated to both baseline VF parameters (MD/PSD) and also the final MD level ($p<0.05$). In this aspect, our results were similar to the OHTS (11) or EMGT study (12), where the initial MD level predicted progression rate in hypertensive glaucoma forms. Additionally in EMGT results, by reducing the IOP with 25%, progression occurred later than in non-treated patients. This could also be the reason for our patients progressing at such a low rate compared to other studies, since medication was constantly changed/ added

to lower the IOP aiming for “the lower, the better” concept. No differences were detected in progression rate between patients with open angle glaucoma and diabetes compared to non-diabetic patients on short term evaluation. MD slope was similar in absolute values and no statistical significance could be attributed, $p=0.55$ (t test). Calculated MD slope at 24 months was -0.19 dB/year in OAG+DM group compared to -0.18 dB/year in OAG group.

In the study group we found correlations between MD slope and mean IOP at baseline; also there was a correlation between visual field decay and the number of topical medication, but when age was controlled the correlation was not significant anymore. Clinically this was associated with a more aggressive treatment from the beginning, in order to prevent a more rapid progression, as previously described by recent literature data where diabetes triggered faster glaucoma progression.

Preliminary analysis of progression at 24 months represented an important step in

monitoring correctly both OAG patients and OAG+diabetes patients, as around this interval we could rule out fast progressing cases (MD slope >-2 dB/year). Limitations of our study were basically the small number of patients and the short term evaluation of glaucoma progression, as all major clinical trials reported data after 4 years minimum (3,5); a clear image of glaucoma progression could have been obtained after a longer period of follow up, as it is known that in the first two years the investigators could only obtain a rough estimate. Still the reason for this evaluation was to rule out fast progressors (MD slope decay >-2 dB/year).

CONCLUSIONS

In conclusion on short term evaluation (24 months), the visual field loss and functional deterioration in open angle glaucoma patients having early/ moderate stages, occurs similarly in the presence or absence of diabetes and no fast progressor was detected in our study during this interval.

REFERENCES

1. Resnikoff S, Pascolini D, Etya'ale D, Kokur I, Pararajasegaram R, Pokharel GP. Global data impairment in the year 2002. *Bull World Health Organization* 2004; 82:844-851.
2. Zahari M, Mukesh BN, Rait JL, Taylor HR, McCarty CA. Progression of visual field loss in open angle glaucoma in the Melbourne Visual Impairment Project. *Clin Experiment Ophthalmol.* 2006; 34:20-26.
3. Leske MC, Heijl A, Hyman L, Bengtsson B, Dong L, Yang Z; EMGT Group. Predictors of long-term progression in the Early Manifest Glaucoma Trial. *Ophthalmology.* 2007;114(11):1965-1972
4. Eid TM, Spaeth GL, Bitterman A, Steinamnn WC: rate and amount of visual loss in 102 patients with open angle glaucoma followed up for at least 15 years. *Ophthalmology.* 2003, 110:900-907
5. Heijl A, Bengtsson B, Hyman L, Leske MC (2009): Natural history of open angle glaucoma. Early Manifest Glaucoma Trial Group. *Ophthalmology.* 2009; 116:2271-2276.
6. Heijl A, Leske MC, Bengtsson B, et al. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Arch Ophthalmol* 2002, 120(10):1268-1279.
7. Hodapp E, Parrish IIRK, Anderson DR: *Clinical decision in glaucoma.* St Louis CV Mosby, 1993; 52-61

Short term evaluation of perimetric progression in patients with open angle glaucoma and diabetes

8. Chauhan BC, Mikelberg FS, Balaszi AG, Le Blanc RP, Leske MR, Trope GE; Canadian Glaucoma Study - Risk factors in the progression of open angle glaucoma. *Arch Ophthalmol*, 2008; 126:1030-1036.
9. Glaucoma evaluation in diabetic patients. Apreutesei NA; Chiselita D; Motas OI; *Rev Med Chir Soc Med Nat Iasi* 2014; 118: 667-674
10. Assessment of visual function under treatment with topical antiglaucoma agents and systemic vasodilators. Clinical study on glaucoma patients. Popa SA; Bucatariu PM; Azoicai D; Costin D; Manole A; Matei M; Merchez; *Rev Med Chir Soc Med Nat Iasi* 2011; 115: 455-459
11. Kass MA, Heuer DK, Higginbotham EJ. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open angle glaucoma. *Arch. Ophthalmol*. 2002; 120(6):701-730.
12. Leske MC, Heijl A, Hyman L, Bengtsson B. Early manifest Glaucoma Trial: design and baseline data. *Ophthalmology* 1999; 106(11):2144-53.

NEWS

THE INCIDENCE OF INVASIVE MRSA INFECTIONS IN GERMANY MAY BE DECLINING

As ECDC mentioned in the Annual epidemiological report for Antimicrobial resistance and healthcare-associated infections 2014 (data for 2012), while MRSA remains a public health priority as the percentage of MRSA isolates is still above 25% in seven of 29 reporting countries, mainly in Southern and Eastern Europe, including Romania (>50%), a recently published study showed that the incidence of invasive MRSA infections in Germany may be declining. MRSA infections in Germany are thought to be mainly healthcare-associated with only a small proportion, which are community-associated or livestock-associated. The notification of MRSA infections is mandatory in Germany since 2009 for microbiological laboratories. A team of researchers conducted a retrospective cohort study in the general population of Germany. The analysis was limited to the period from 2010 to 2014. Data of notifications of invasive MRSA infections for the above-mentioned period were extracted from the national surveillance database at the Robert Koch Institute as collected up to 1 March 2015 (Walter J, Haller S, Blank H et al. Incidence of invasive methicillin-resistant *Staphylococcus aureus* infections in Germany, 2010 to 2014. *EuroSurveill*. 2015; 20(46):pii=30067. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2015.20.46.30067>).

Odette Popovici

THE ACUTE SCROTUM IN CHILDREN

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THE ACUTE SCROTUM IN CHILDREN (Abstract): The acute scrotum syndrome is a medical-surgical emergency and the recognition of this condition by both healthcare professionals and the general population may result into the patients' coming in earlier for medical examination and into the preservation of the gonad in case of torsion. The purpose of this retrospective analytical research is to point out specific epidemiological aspects in pediatric patients suffering from acute scrotum, and to review the existing diagnosis and treatment options. The study included 208 patients, of whom 16 with vanishing testis and 192 with acute scrotum (torsion of testis 25.5%, torsion of the hydatid of Morgagni 68.2%, epididymo-orchitis 5.2%). The torsion of the hydatid of Morgagni occurs in boys with a mean age of 10 years and it involves both testes equally, whereas the torsion of testis usually occurs around the age of 13 and is twice more common in the left gonad. Another significant difference between the two conditions is the inflammatory syndrome, which occurs in 45.4% of the children with torsion of testis versus only 18.2% in the torsion of hydatid. Only one out of six testes torqued during the neonatal period could be saved (16.6%); the gonad preservation rate was as high as 68.2% in the group of patients with testis torsion occurring outside the neonatal period. These alarming data are accounted for by the non-recognition of the severity of the condition and by the delayed surgical therapy, which occurs on the average 20 hours after the testis torsion has set in. If the asepsis and antisepsis standards are observed, patients with torsion of the hydatid of Morgagni or torsion of testis require neither fluid sampling from the tunica vaginalis for culture, nor antibiotic therapy. **Keywords:** ACUTE SCROTUM, TESTICULAR TORSION, TESTICULAR APPENDAGE, EPIDIDYMO-ORCHITIS.

The acute scrotum syndrome refers to the sudden onset of pain, swelling and other inflammatory phenomena in a hemiscrotum, which may be caused by conditions such as orchiepididymitis, torsion of testis or appendix testis, testicular hematoma due to local injury or hematocele due to adrenal hemorrhage during the perinatal period, testicular tumor, strangulated inguinal or scrotal hernia, acute scrotal edema, Henoch-Schönlein purpura. Strangulat-

ed inguinal hernia and testis torsion are surgical emergencies, whereas orchiepididymitis may only require medical therapy. Scrotal injury with hematoma or incarcerated hernia may be diagnosed according to anamnesis, whereas Doppler ultrasound scanning may differentiate the inflammatory phenomena due to orchiepididymitis from the absence of blood flow due to testis torsion (1). A detailed history, through physical examination of the genitalia and

medical imaging examinations such as Doppler ultrasound scanning, and sometimes scintigraphy or IRM, usually allow a preoperative differential diagnosis. Nevertheless, even the slightest diagnosis doubt requires surgical examination designed to rule out testis torsion as the cause of the acute scrotum and to save the gonad (2,3,4). The purpose of this research is to emphasize specific epidemiological aspects in pediatric patients suffering from acute hemiscrotum syndrome, and to review the existing diagnosis and treatment options in a regional Romanian center.

MATERIAL AND METHODS

We have conducted a retrospective analytical study on 257 patients hospitalized and treated in the Pediatric Surgery Clinic of the "Sfânta Maria" Children's Hospital of Iași over a period of about 3 years (January 2012 – February 2015). Our hospital is the only tertiary hospital in the Moldova area, which treats pediatric patients coming from 7 counties. The patient data were retrieved from the hospital's computerized database, after entering the disease code N44, which stands for "Torsion of Testis". The system generated 257 patients encoded with this diagnosis, from a total number of 4816 diagnoses. Only 208 of them were eligible for our research, the remaining 49 being excluded as their diagnoses did not correspond to the aim of our study. We carried out an analysis and statistical processing of the patients' demographic data, diagnosis on hospital discharge, age on the first hospitalization, number of hospitalization days, personal history, paraclinical tests and examinations, treatment method, operating technique, subsequent evolution, pathology findings. All patients were seen by at least one specialist doctor, routine complete blood counts, inflammatory tests

(ESR, Fibrinogen) and urinalysis were performed. Sometimes, additional examinations were conducted, i.e. abdominal and scrotal ultrasound scanning, Doppler ultrasound scanning and even CT in uncertain cases. The best course of care adopted in our clinic recommends a surgical procedure in all the acute hemiscrotum syndrome cases, in whom suspected testis torsion cannot be ruled out by clinical and paraclinical means, or in whom the painful symptoms due to torsion of the hydatid of Morgagni do not subside within a few hours from hospitalization. The statistical analysis was conducted using SPSS for Windows and Microsoft Excel. The data were reported as means $\pm$ standard deviations. The differences between groups were analyzed using the t Student test, and the level of statistical significance was $p \leq 0.05$.

RESULTS

131 of the 208 patients included in the study were finally diagnosed with torsion of the hydatid of Morgagni, 65 with torsion of testis, 10 with acute orchiepididymitis and 2 with post-traumatic scrotal hematoma. Excluding the 16 patients who came in for congenital undescended testicle, which postoperatively proved to be vanishing testis, among the acute scrotum group the testis torsion rate was 25.5%, the rate of torsion of the hydatid of Morgagni was 68.2% and orchiepididymitis 5.2%.

After group analysis, we noted that most of the patients came from urban areas (1.33 ratio). The overall mean age of the patients was 9.66 ± 4.47 years (Fig. 1). 44 of the 65 children finally diagnosed with testis torsion came in for acute scrotum syndrome at a mean age of 13.01 ± 3.93 years, 5 came in immediately after birth and were diagnosed with neonatal testis torsion and 16 patients came in for congenital undes-

cended testicle at the mean age of 3.10 ± 3.00 years; their postoperative diagnosis was vanishing testis. As concerns the mean age on consultation for acute hemiscrotum syndrome, a comparison between the 131 patients with torsion of the hydatid of Morgagni (9.82 years) and the 49 with torsion of testis (11.68 years), reveals a difference of about 2 years, which is statistically significant ($p=0.001$). The differ-

ence increases if we exclude from the second group the five patients who came in during the neonatal period, i.e. 9.82 versus 13.01 years. The torsion of the hydatid of Morgagni occurred on the left side in 67 patients, on the right side in 61 patients and on both sides in 3 patients, whereas the torsion of testis impaired 21 patients in the right side, 42 patients on the left side and 2 patients on both sides.

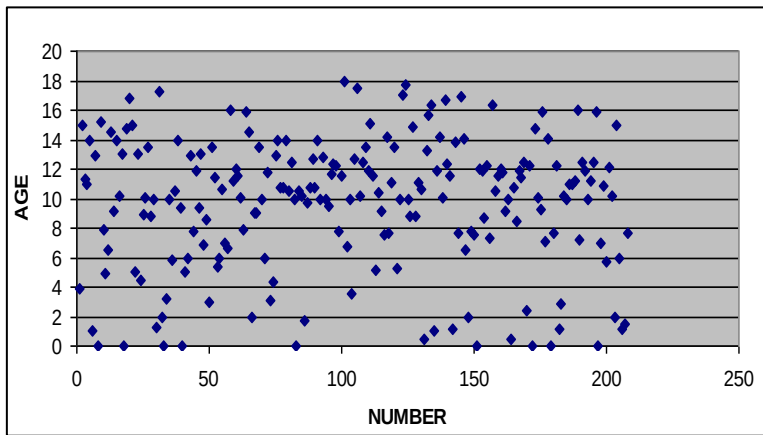


Fig. 1. Distribution of patients by age

The analysis of the personal history of the 131 patients with torsion of the hydatid of Morgagni revealed a prior injury in 7 of them and an intercurrent respiratory condition in 5 other children. Associated conditions were detected in 28 children, 14 of whom suffered from uro-genital conditions. The mean period of time elapsed between symptom occurrence and the patients' seeking medical assistance was 21 hours (between one hour and 7 days). In 44 of the patients the symptoms were edifying, and the doctor on call did not request medical imaging tests, whereas in the other 87 children an abdominal and scrotal ultrasonography was performed. The lab tests revealed an inflammatory syndrome (high leukocytosis, ESR and Fibrinogen levels)

in 24 children (18.2%) and nutritional anemia in 9 of them (6.8%). Only three patients were prescribed conservative treatment consisting of bed rest, non-steroidal anti-inflammatory drugs and cold compressions on the aching spot, and they were fully recovered. The other patients underwent surgery under general anesthesia, which consisted of hemiscrotum exploration, removal of the tormented hydatid of Morgagni (Fig. 2).

A biopsy of the tunica vaginalis was performed in 9 patients, and fluid was sampled in 55 other patients for culture and antimicrobial susceptibility testing. The culture was negative in all the 55 cases. After the surgery, 59 patients were given antibiotic therapy, namely cephalosporins

or beta-lactams. The postoperative evolution of four patients was marked by the occurrence of a scrotal hematoma, three of whom underwent a second surgery to remove it. Another patient needed a second surgery for postoperative scrotal abscess. The other patients recovered rapidly, and they were discharged, on the average, 2.6 days after the surgery. No pathology tests could be conducted on the removed hydatid of two of the patients due to the fact that the removed mass was too small. In all the other patients, the microscopic examination revealed signs of stasis, inflammation, hemorrhage, hemorrhagic necrosis (Fig. 3).



Fig. 2. Torsion of the hydatid of Morgagni – intraoperative aspect

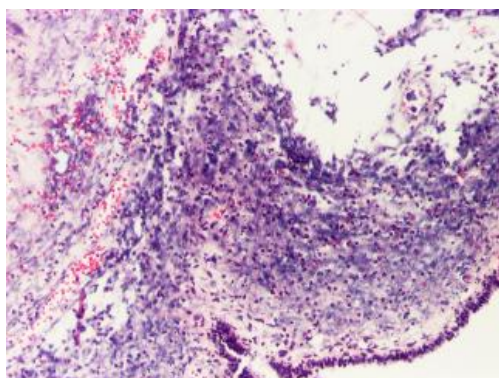


Fig. 3. Hydatid of Morgagni – haemorrhagic necrosis and leukocytic exudate – HEx100

Three of the 44 patients with torsion of the testis who came in for acute hemiscrotum syndrome outside the neonatal period had suffered prior injury. Associated conditions were detected in 8 children, as 6 of them were also suffering from uro-genital diseases. The mean period of time elapsed between symptom occurrence and the patients' seeking medical assistance was 20 hours (between one hour and 11 days). The lab tests revealed an inflammatory syndrome in 20 children (45.4%) and nutritional anemia in 3 of them (6.8%). Surgery was performed without preoperative imaging tests in only 6 patients, whereas an abdominal and scrotal ultrasonography was performed in the other 38 patients. Since it is not readily available in our hospital, Doppler ultrasonography was conducted preoperatively only in 6 patients, and postoperatively in 10 patients. A pelvic CT scan was performed in an adolescent suspected of testis tumor, but the postoperative pathological examination set the diagnosis of old testis torsion, with total glandular necrosis (Fig. 4). The testis was surgically approached through the scrotum in all the patients, the testis was detorted (between 180° and 1080°), the spermatic cord was injected with xylocaine and, depending on the recoloring degree, the surgeon decided to keep or to ablate the gonad (Fig. 5). Orchiectomy was performed in 7 patients due to the clearly necrotic gonad (the mean period of time before going to see a doctor was 4.4 days for these patients), whereas in other two patients orchiectomy was carried out after 48 hours (on second look). In the other 35 patients, the doctors decided to keep the gonad on condition of its regular ultrasonographic follow-up, and the disease progressed (one and three-month follow-up) in other 5 patients. As concerns the 14 patients in whom the dis-

ease progressed (31.8%), 7 of the group of 21 were living in rural areas and 7 of the group of 23 were living in an urban environment. Thus, there were no significant differences as concerns the patients' living environment. Overall, after detorting, the three-month follow-up revealed that 30 patients (68.2%) recovered. Orchidopexy was performed on the impaired side in the patients in whom the gonad was preserved, and contralateral orchidopexy was preferred for 7 patients. Two patients required a second surgery, one for the excision of a post-operative hematoma and the other for pus draining. The mean hospitalization duration was 3.7 days for the first surgery.

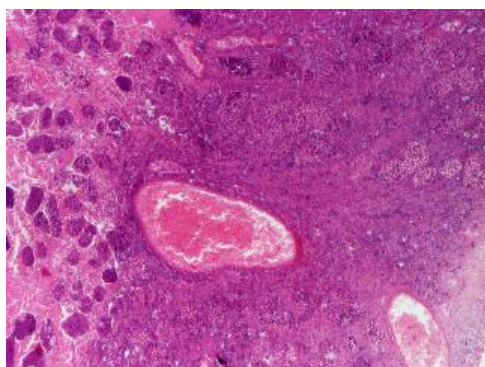


Fig. 4. Testicular torsion - haemorrhagic necrosis – Hex40



Fig. 5. Testicular torsion – intraoperative aspect

Two of the 5 patients with neonatal testis torsion, who came in immediately after birth for acute scrotum suffered from torsion on the right side, two on the left side and one on both sides; orchiectomy was performed in 2 children on the first surgical procedure as there were no doubts about the ischemic necrosis diagnosis, whereas in a patient the gonad was initially preserved but it was finally removed two days later (on second look). Contralateral orchidopexy was also performed in all these patients. The right testis of the child with bilateral torsion was removed on account of the advanced necrosis, whereas his left testis was detorted and fixed, but it later progressed to testicular atrophy. In another patient the testis was detorted and the spermatic cord was injected with xylocaine. The testis was preserved and the 3-month follow-up ultrasonography revealed the patient's recovery. Thus, only one out of six testes could be saved (16.6%).

Fluid was sampled from the testicular vaginal tunic of 29 of all the 49 patients with acute hemiscrotum syndrome due to testis torsion, and the culture was positive in 3 children younger than 6 months; 34 children were administered broad-spectrum antibiotics.

Ten patients, with a mean age of 9.97 years (two newborns, one 5 month infant and 7 children older than 10 years), with acute scrotum syndrome were diagnosed with acute orchiepididymitis intraoperatively. Fluid from the tunica vaginalis was sampled and the result was positive in five of them. All the patients were administered antibiotic therapy. The patients were examined for genitourinary tract abnormalities, detected in 3 of them, and urinary infections, present in two patients. The evolution was poor in a single child with cicatricial phimosis, recurrent urinary in-

fections and postoperative scrotal abscess, who required orchiectomy.

Two boys exhibited right vanishing testis, one suffered from bilateral torsion and 13 suffered from left testis torsion. The congenital undescended testicle was examined by inguinal approach in 9 children and by laparoscopic approach in 7; biopsy of the blind-ending deferent duct was performed in 12 patients (Fig. 6).

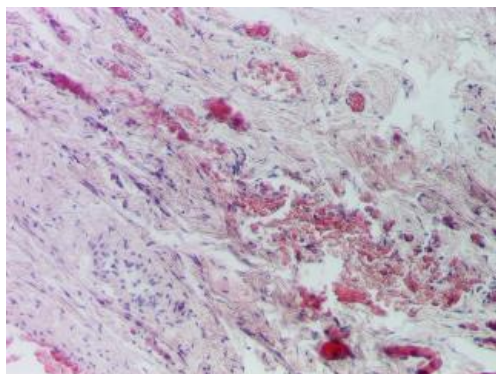


Fig. 6. Vanishing testis –connective-vascular tissue– HEx100

DISCUSSIONS

The medical technological breakthroughs achieved lately have led to the decrease of the number of surgical procedures performed in patients with acute scrotum syndrome. Different studies have proven that only 14-38% of the patients with acute scrotum suffered from testis torsion, despite the physical examination and their personal history that would indicate surgery (5). Our research revealed a 25.5% testis torsion incidence rate, a 68.2% Morgagni hydatid torsion incidence rate and a 5.2% orchiepididymitis incidence rate.

Torsion of appendix testis, more precisely of the hydatid of Morgagni and more rarely of the appendix epididymis, is the

most common cause of acute scrotum syndrome in children. The hydatid of Morgagni is an embryonic remnant originating in the Müllerian duct, attached to the upper testis pole and occurring in about 90% of the patients. The symptoms evocate an acute scrotum syndrome, the pain sets in progressively, unlike in torsion of testis, and is less sharp, and is sometimes located only in the upper testis pole, where the torted hydatid may be palpated and viewed (blue dot sign). In order to avoid about two thirds of the surgical procedures that are not absolutely necessary each patient should undergo medical imaging tests, more precisely Doppler ultrasound scanning. Ultrasonography sometimes reveals the hydatid as a hypoechogenic mass, which is highly vascularized due to inflammation, and a reaction hydrocele may also occur. The Doppler ultrasound scan may reveal and quantify the blood flow in the testis and the scrotum, and may provide information about the structural integrity of the gonad. The treatment may be conservative and consist of rest, ice packs, scrotum lifting, anti-inflammatory and analgesics. If the diagnosis is uncertain, the pain very sharp or does not subside further to treatment, surgery is recommended for the removal of the necrotic hydatid (6). Unfortunately, since Doppler ultrasound scans could not be performed on the patients hospitalized in our clinic and since there were no well established therapeutic protocols, far too few children could benefit from this examination method and therefore only three of the patients with torsion of the hydatid of Morgagni received conservative treatment, which considerably increased the surgical procedure rate. Nevertheless, this is not a bad approach, since most authors recommend immediate surgery if there is even the slightest doubt

about the diagnosis, all the more so as any medical imaging technique may have false negative results and thus delay the surgical procedure that may save a torsted testis (2,3).

Torsion of testis is a surgical emergency that may occur at any age and, if improperly treated, may lead to infertility and psychological impairment. The annual incidence rate of this condition is 1/4000, and about 61% of all the patients are younger than 25 years, and exhibit bimodal age distribution, namely during the neonatal period and around the age of 13 years (7). Our research supports these data, as all the patients younger than 1 year suffered from testis torsion. When comparing the mean age at which the patients came in for acute scrotum, we noted a significant difference of about 3 years between the two groups, namely the 131 patients with torsion of the hydatid of Morgagni (9.82 years) and the 44 patients with torsion of testis (13.01 years). Similar studies have also reported older age of patients with torsion of testis than of patients with torsion of the hydatid of Morgagni (8).

In children, testicular torsion usually occurs in the absence of any predisposing factor, since a prior scrotal injury or intense physical effort was only reported in 4-8% of the cases (9). In our case, a prior injury was reported only in 5.3% of the children with torsion of the hydatid of Morgagni and in 6.8% of the children with testis torsion. Other possible triggering factors are the sudden testis volume increase during puberty, congenital undescended testicle, testicular tumors, low temperatures associated with an exaggerated cremasteric reflex, presence of a long intrascrotal spermatic cord (9). Whereas appendix testis torsion seems to affect both sides equally (27 left versus 21 right), left

side impairment by torsion of testis was twice more common than right side impairment, which was also reported by other literature studies (8). Another significant difference between the two conditions is the occurrence of the inflammatory syndrome in 45.4% of the children with torsion of testis versus 18.2% in those with torsion of hydatid. Other studies also suggested that leukocytosis and the NLR (neutrophil to lymphocyte ratio) are predictive of inflammatory processes and also torsion of testis (10,11).

Detorting during the first 6 hours is associated with over 90% chances of saving the testis, whereas the same procedure applied more than 24 hours from the event only saves less than 10% of the testes (12). Surgery is sometimes delayed due to lack of medical education, to children living in disadvantaged families who do not seek medical assistance, to long distances to the nearest hospital or sometimes to the failure of the first doctor who examines the child to recognize the condition. In our research, the mean period of time elapsed between symptom occurrence and the patients' seeking medical assistance was 21 hours for children with torsion of the hydatid of Morgagni and 20 hours (between one hour and 11 days) for those with torsion of testis, which explains the testicular preservation rate of only 68.2%. In our research, the patients' living environment did not influence in any way their postoperative evolution. The surgeons usually decides whether to preserve or remove the gonad during the surgical procedure, depending on the time elapsed from symptom occurrence, on the degree of torsion, on the color of the testis after detorsion, and possibly after the spermatic cord has been injected with xylocaine. If the black-purplish color persists, this indicates irreversible glandular necro-

sis and recommends removal, whereas if the testis becomes pink again, it should be preserved and orchidopexy should be done. The torsed testis preservation rate varies between 58 and 75.7% in various studies (13,14). Most authors recommend bilateral orchidopexy after detorsion, in order to prevent any reoccurrence (15), but in the absence of therapeutic protocols this was not complied with throughout our study. Orchidopexy is done by fastening the testis against the scrotal wall using two or three suture points with nonabsorbable stitches crossing the tunica albuginea. Although it has been argued that this technique may trigger the production of antispermatic antibodies, this has not been accurately proven and, at any rate, the torsion reoccurrence risk seems higher (16).

Originally described by Taylor in England, in 1897, and then 50 years later by Campbell in North America (17,18), perinatal testicular torsion is rare yet controversial event as to its etiology, clinical form, management and long-term sequelae. Perinatal testicular torsion includes antenatal torsion and torsion occurring during the first 30 days after birth, and it makes up about 12% of testis torsions in children (10.2% in our research). The possible etiopathogenic theories include difficult delivery, breech presentation, multiparity, high birth weight and exaggerated cremasteric reflex (19). Prenatal torsion has minimum clinical symptoms and no discomfort, whereas postnatal torsion has significant clinical symptoms, such as pain and acute inflammatory phenomena in a previously healthy testis. Since antenatal ultrasonography does not have high sensitivity to this abnormality, antenatal torsion is usually detected on the routine newborn checkup and it is usually too late to save the gonad. Therefore, the management of this condi-

tion is highly controversial. Some authors only recommend clinical and ultrasound monitoring and avoidance of surgical procedures, whereas others suggest immediate scrotal or inguinal exploration and contralateral orchidopexy (20). According to other authors, if the torsion occurs much before the birth (the inflammatory signs on the hemiscrotum are discrete), surgery can wait and the patient may receive conservative treatment or elective orchiectomy and contralateral orchidopexy will be done; if the torsion occurs at birth or after birth, surgery is a major emergency and is to be done even in the absence of any medical imaging tests (21). The advocates of conservative treatment rely on the low incidence of contralateral torsion and support the avoidance of any anesthetic and surgical risks during the neonatal period. Moreover, if the parents are correctly informed, they will bring the child to the emergency room on the first signs and symptoms of torsion of testis, which means that surgery may be done on time. In a study conducted on 48 cases of bilateral perinatal testicular torsion, despite the prompt surgical intervention in 46 of these patients, only 3 testes exhibited a blood flow on postoperative Doppler ultrasonography (20). In our research, only one patient exhibited signs of recovery at the three-month follow-up, and the gonad had to be removed due to advanced necrosis in all the other cases.

If the asepsis and antisepsis standards are observed, patients with torsion of the hydatid of Morgagni or torsion of testis confirmed and surgically treated require neither fluid sampling from the testicular vaginal tunic for culture, nor antibiotic therapy. Nevertheless, fluid was sampled from the tunica vaginalis of 55 patients with torsion of the hydatid of Morgagni, for culture purposes, and the results were

negative in all these cases; furthermore, 59 patients were administered intravenous antibiotic therapy. Only one patient living in a disadvantaged social environment and who did not observe the postoperative hygiene requirements developed a scrotal abscess 5 days after the surgery and required a second surgical procedure. As for the patients with torsion of testis, only 3 of the 30 samples collected were positive, all three in infants under the age of 6 months; on the other hand, 34 patients were administered broad-spectrum antibiotic therapy. In our opinion, according to these data, fluid sampling from the testicular vaginal tunica is only necessary for patients with orchiepididymitis or strong inflammatory phenomena. Prophylactic antibiotic therapy is recommended only to infants with acute scrotum who underwent surgery.

Orchiepididymitis is the most common cause of acute scrotum syndrome after puberty, with an incidence peak at the age of 40-50 years, but it may also occur in babies. In our research, the incidence rate of this condition was 5.2% versus 9% or 16% in other studies, which also included patients older than 18 years (8,22), and the disease affected two categories of patients, namely neonates and adolescents. Surgery was performed in all the patients, who were administered antibiotic therapy. The evolution was positive in 9 out of 10 cases. The most common etiology is urinary tract

infection related to possible associated malformations, hematogenous infection or scrotal injury. The treatment is conservative if we are absolutely certain about the diagnosis and it consists of analgesics, anti-inflammatory drugs, ice pack and antibiotic therapy, under ultrasound scanning and biological monitoring. Nevertheless, if there is even the slightest doubt surgery is to be done in order to rule out testis torsion as a cause of acute scrotum.

CONCLUSIONS

Torsion of the hydatid of Morgagni occurs in boys at the approximate age of 10 years and it affects both testes equally, whereas torsion of testis usually occurs at the age of 13 and affects twice more often the left gonad. Another significant difference is the inflammatory syndrome, which occurs in 45.4% of the children with torsion of testis versus only 18.2% of those with torsion of hydatid. Only one out of six tortured testes could be saved in the neonates (16.6%), whereas in the group of patients with torsion of testis that were not neonates, the gonad preservation rate was 68.2%. These data are accounted for by the non-recognition of the severity of the condition and by the delayed surgical therapy. Patients with torsion of the hydatid of Morgagni or torsion of testis require neither fluid sampling from the tunica vaginalis for culture, nor antibiotic therapy.

REFERENCES

1. Agrawal AM, Tripathi PS, Shankhwar A, Naveen C. Role of ultrasound with color Doppler in acute scrotum management. *J Family Med Prim Care*. 2014 Oct-Dec;3(4):409-12.
2. Molokwu CN, Somani BK, Goodman CM. Outcomes of scrotal exploration for acute scrotal pain suspicious of testicular torsion: a consecutive case series of 173 patients. *BJU Int*. 2011;107(6):990-3.
3. Baker LA, Sigman D, Mathews RI, Benson J, Docimo SG. An analysis of clinical outcomes using color doppler testicular ultrasound for testicular torsion. *Pediatrics*. 2000;105(3 pt 1):604-7.

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4. Wu HC, Sun SS, Kao A, Chuang FJ, Lin CC, Lee CC. Comparison of radionuclide imaging and ultrasonography in the differentiation of acute testicular torsion and inflammatory testicular disease. *Clin Nucl Med*. 2002;27(7):490-3.
5. Koester MC. Initial evaluation and management of acute scrotal pain. *J Athl Train*. 2000 Jan;35(1):76-9.
6. Srinath H. Acute scrotal pain. *Aust Fam Physician*. 2013 Nov;42(11):790-2.
7. Cummings JM, Boullier JA, Sekhon D, Bose K. Adult testicular torsion. *J Urol*. 2002;167(5):2109-10.
8. Mohammad Kazem Moslemi, Sharifeh Kamalimotlagh. Evaluation of acute scrotum in our consecutive operated cases: a one-center study. *Int J Gen Med*. 2014; 7: 75–78.
9. Seng YJ, Moissinac K. Trauma induced testicular torsion: a reminder for the unwary. *J Accid Emerg Med*. 2000;17(5):381-2.
10. Thomsen M, Ingebrigtsen TS, Marott JL, Dahl M, Lange P, Vestbo J, et al. Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. *JAMA*. 2013; 309:2353–61.
11. Güneş M, Umul M, Altok M, Akyuz M, İsoğlu CS, Uruc F, et al. Predictive role of hematologic parameters in testicular torsion. *Korean J Urol*. 2015 Apr; 56(4):324-9.
12. Sessions AE, Rabinowitz R, Hulbert WC, Goldstein MM, Mevorach RA. Testicular torsion: direction, degree, duration and disinformation. *J Urol* 2003; 169: 663-5.
13. Zhao LC, Lautz TB, Meeks JJ, Maizels M. Pediatric testicular torsion epidemiology using a national database: incidence, risk of orchiectomy and possible measures toward improving the quality of care. *J Urol* 2011; 186: 2009-13.
14. Lee SM, Huh JS, Baek M, Yoo KH, Min GE, Lee HL, et al. A nationwide epidemiological study of testicular torsion in Korea. *J Korean Med Sci*. 2014 Dec;29(12):1684-7.
15. Lopez RN, Beasley SW. Testicular torsion: potential pitfalls in its diagnosis and management. *J Paediatr Child Health* 2012;48:E30–32.
16. Arap MA, Vicentini FC, Cocuzza M, Hallak J, Athayde K, Lucon AM, et al. Late hormonal levels, semen parameters, and presence of antisperm antibodies in patients treated for testicular torsion. *J Androl*. 2007 Jul-Aug;28(4):528-32.
17. Taylor MR. A case of testicle strangulated at birth; castration; recovery. *BMJ* 1897;1:458.
18. Campbell MF. Torsion of the spermatic cord in the newborn infant. *J Ped* 1948;33:323.
19. Leach GE, Masih BK. Neonatal torsion of the testicle. *Urology* 1980;16:604-5.
20. Baglaj M, Carachi R. Neonatal bilateral testicular torsion: a plea for emergency exploration. *J Urol* 2007;177:2296-9.
21. Cuervo LJ, Grillo A, Vecchiarelli C, et al. Perinatal testicular torsion: a unique strategy. *J Pediatr Surg* 2007;42(4):699-703.
22. Mushtaq I, Fung M, Glasson MJ. Retrospective review of pediatric patients with acute scrotum. *ANZ J Surg*. 2003;73(1–2):55–58.

PROLONGED FEVER WITHOUT OBVIOUS ETIOLOGY FINALLY DIAGNOSED AS HIGH-GRADE SOFT TISSUE SARCOMA

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PROLONGED FEVER WITHOUT OBVIOUS ETIOLOGY FINALLY DIAGNOSED AS HIGH-GRADE SOFT TISSUE SARCOMA (Abstract): Sarcomas represent a challenge to clinicians as they are rare and diagnosis is often delayed. Soft tissue sarcomas are relatively uncommon cancers, the incidence is estimated about 1% from all malignant tumors. Due to aggressive biological behavior of pleomorphic undifferentiated sarcoma diagnosis must be made as quickly as possible. MRI is the standard imaging modality for investigating possibly malignant masses. Excisional biopsy appears to be necessary for differentiating soft tissue sarcoma from expanding hematoma. FDG PET scans may be helpful in specific circumstances (as was the situation in our case, because the lesion is composed from two components: an area without precise edges and a necrotic-cystic area). **Keywords:** SOFT TISSUE SARCOMA, FDG PET SCAN, PROLONGED FEVER, EXCISIONAL BIOPSY.

The large majority of soft tissue tumors are benign, with a very high cure rate after surgical excision. Soft tissue sarcoma (STS) incidence is estimated about 1% of malignant tumors, life-threatening being the main problem, the diagnosis and treatment representing a significant challenge due of more than 50 histological subtypes of soft tissue sarcomas which often have specific characteristics in clinical presentation, prognosis and treatment (1).

Undifferentiated high grade sarcomas are usually present in patients aged over 40 years, predominance beings around the age of 60-70 years, rare examples may be encountered in adolescents and young adults. Preponderance it's a little bit higher in men versus women, approximately 1.2:1(2). STS distribution according to the anatomic area as follows: head and neck 8.9%, trunk

17.9%, upper extremities 13.1%, lower extremities 46.4%, retroperitoneum 12.5%, mediastinum 1.3% (3). In the extremities, STS it's presenting most often as a painless lump or swelling, usually 5-10 cm in diameter, located in muscle (4). In order to be able to differentiate between various types of sarcomas and discriminating through the newly diagnosed low-grade and high-grade sarcomas, a high sensitivity it's required and this may be achieved by combining metabolic and morphological information of FDG PET/CT imaging (5). These combined images have been already used for multiple purposes: biopsy guidance, response assessment, grading, and follow up in STS (6).

Among the most often used systems for classification we notice: French grading and the National Cancer Institute grading,

which are based on histologic type and subtype, tumor necrosis, and mitotic activity. Further evolution to metastatic disease is given by histological grading which is also an important prognostic factor (7).

CASE REPORT

A 67 years old Caucasian female, without significant medical history, was admitted with prolonged fever (up to 39°C), chills, sweats, diffuse myalgia, fatigue, persistent inflammatory syndrome, weight loss (10 kg in last 5 months) and a recent appearance of a pain in left thigh without swelling. The medical investigations performed before admission: thorax scanner, bone scintigraphy, abdominal ultrasound and transthoracic cardiac echography were negative. The patient was treated during 15 days with corticosteroids (Medrol 12 mg/d), but without symptomatic improvement.

Clinical examination reveals pain on deep palpation in the left trochanter without local swelling. Vital parameters were normal except the fever.

Laboratory investigations show the following results: CRP 148mg/L, hemoglobin 10,2g/dL, ferritinemia 840,4μg/L (normal value between 30 to 400), serum iron 16μg/dL, hypoalbuminemia 20,9g/L. Bacteriological analyzes of urine and blood were negative.

Due to the fact that the patient began to invoke a pain in left thigh we chose to do some investigations to discover the etiology. A pelvis and femur radiography reveals no bone lesions. A bone scintigraphy shows on the precocious images an intake at the soft tissue of the left thigh. We continued investigations with a hip ultrasound which revealed a heterogeneous formation of 112 mm on 40mm which occurs vascularized. We followed with an MRI exams which detected a lesion of 10 cm containing two

components: a fleshy area without precise edges and a necrotic-cystic area, suspicious of malignancy (rhabdomyosarcoma), as illustrated in the following two figures:

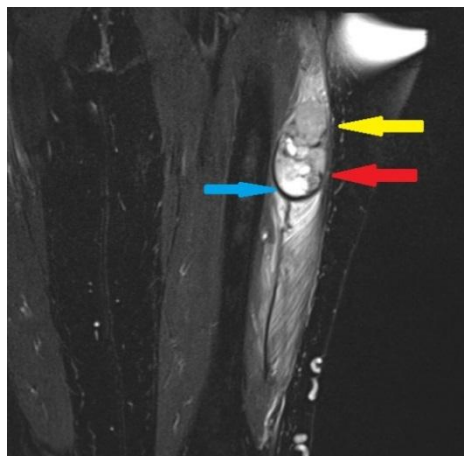


Fig. 1. MRI of the thigh: coronal cut, T2-weighted. The lesion at the vastus lateralis muscle occupying a major part of this muscle, that seems not to cross the vast intermediate aponeurosis, showing a double signal: the cranial part appears fleshy (yellow arrow) and caudal part appears necrotic-cystic (red arrow) well delimited by a fibrous membrane (blue arrow).

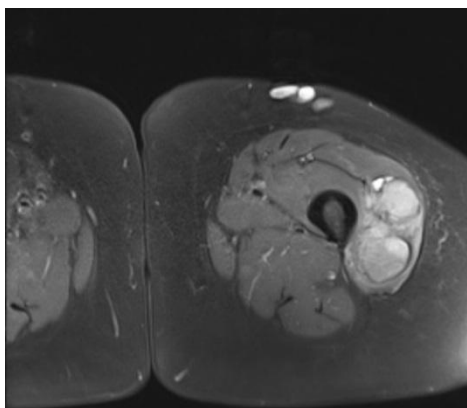


Fig. 2. MRI of the thigh: axial cut, T2-weighted. The lesion in the vastus lateralis muscle occupying a major part of this muscle.

We have continued the investigations with a FDG PET scan which revealed us a voluminous lesion very hypermetabolic. We proceeded to the surgical excision of the mass which led us to the diagnosis of soft tissue sarcoma.

DISCUSSION

Unexplained prolonged fever is characterized by a higher temperature than $38,3^{\circ}\text{C}(101^{\circ}\text{F})$ lasting more than 3 weeks for no apparent reason after clinical examination and routine tests.

The main causes of prolonged fever (except patients with a known HIV positive serology) are: infections (20 to 35% of cases), malignancies (10 to 15% of cases), autoimmune conditions (20 to 30% of cases) and miscellaneous (5 to 15% of cases) (8). In rare cases, soft tissue sarcoma can cause general symptoms such as: fever, chills, sweats, weight loss, fatigue. Soft tissue sarcoma in its early stages may grow without any signs or symptoms, pain or soreness appear when it presses against nearby nerves and muscles.

The first imaging test is a X-ray, followed by an MRI which is the most helpful test to image soft tissue tumors as it provides very important information about the mass such as the size, location, and proximity to neurovascular structures but the diagnosis of a tumor cannot be made by MRI alone. MRI cannot be done to patients with metal implants or pacemakers, in those cases a CT scan may be an option.

Rhabdomyosarcoma is part of tumors of the musculoskeletal tissue for which the performing of a ^{18}F -fluorodeoxyglucose (FDG) PET-CT is indicated in order to stage the high-grade sarcomas (9). Considering the suspicion of rhabdomyosarcoma and that the lesion has several components, to avoid the risk of false negative result at

biopsy we opted to continue investigations with a FDG PET scan due its direct relationship to tissue metabolism. The highest FDG uptake areas in a tumor reflect the most biologically aggressive areas with the probability of malignant behavior.

Below we present PETAC axial and axial CT images which have been fused to obtain a third image which shows a voluminous lesion very hypermetabolic.

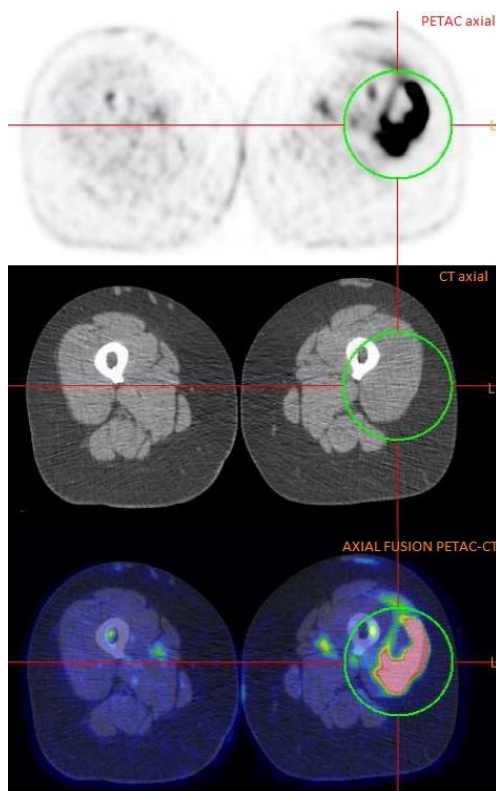


Fig. 3. Fusion between Positron Emission Tomography with Carbon-11 Acetate axial and CT axial images.

The standard uptake value(SUV) used in Flourodeoxyglucose (FDG) imaging, has the capability in helping to distinguish between benign and malignant lesions, high SUV uptake may predict for an aggressive

histology. Because the lesion was intense hypermetabolic (SUVmax=49,6) we decided a total excision of the mass.

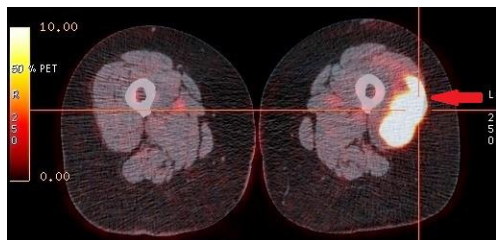


Fig. 4. Fused axial PET/CT images showed tracer uptake in a bulky soft-tissue mass in the left vastus lateralis muscle as indicated by red arrow

In case of mass suspicious of malignancy, the FDG PET image used in conjunction with other imaging can guide the surgeon to the most appropriate area to get biopsy tissue for diagnosis because the most biologically aggressive areas of a tumor have the highest FDG uptake (10).

Only the biopsy can provide a specific histological diagnosis of pleomorphic sarcoma which cannot be obtained by radiologic imaging. There are three procedures to obtain biopsy: fine needle aspiration (FNA) is not recommended as a primary diagnostic modality, core needle biopsy but several cores should be taken to maximize diagnostic yield and excisional biopsy.

After analyzing of surgical fragments, the anatomopathological result concludes the following diagnostic: Soft Tissue Sarcoma undifferentiated High-Grade 3 in the classification FNCLCC (Federation Nationale des Centres de Lutte le Cancer).

When the diagnosis of sarcoma is made, it is important to determine if a tumor is isolated (localized) or has spread (metastatic) by way of PET scan, which nowadays has become a routine exam in the extension evaluation of STS. Sarcomas can develop

distant metastases and the most common site is the lung with the observation that if a pulmonary nodule is discovered after the patient has been diagnosed with a sarcoma, this one may be a metastasis of sarcoma. Rarely metastases may occur in the skin, soft tissues, liver, and lymph nodes (11).

Once the diagnosis of Soft Tissue Sarcoma has been confirmed, an individual treatment plan is made for each patient. Sarcoma treatment requires a multimodality approach and hence a team of physicians will participate in a patient's care. There are essentially three main types of treatment: surgery, radiation, chemotherapy.

Adjuvant chemotherapy is among the treatments that can reduce the risk of developing subsequent systemic metastasis in addition to achieving a good local control in case of high grade sarcomas, greater than 5cm (12).

In our case, after surgical excision of the tumor (with healthy margins of resection) were not found metastases in tests performed. Due of large size of the tumor (10 centimeters) and because there were no metastases found corroborated with a good health condition of the patient, we had considered appropriate to administrate an adjuvant chemotherapy by Adriamycin 3 sessions of intravenous continuous infusion over 3 days every 3 weeks followed by a complement of radiotherapy.

CONCLUSIONS

In prolonged fever of unknown origin we can expect sometimes an infrequent diagnosis, so the presumptive diagnosis should not remain obtuse, but must keep a wide vision to avoid missing the rare diagnosis and particularly aggressive as in our case. Diagnostic steps must be well structured in order to reach the diagnostic as quickly as possible to discover the disease in its earliest stages

to improve the survival prognosis.

While some imaging scans such as CT and MRI isolate organic anatomic changes in the body, PET is capable of detecting areas of molecular biology detail (even prior to anatomic change).

The high diagnostic accuracy of FDG-PET and PET/CT has been well documented for several neoplasms, with a consequent rapid increase in the utilization and acceptance of PET imaging in the clinical setting.

REFERENCES

1. Fletcher CDM, Rydholm A, Singer S, Sundaram M, Coindre JM. Soft tissue tumors: Epidemiology, clinical features, histopathological typing and grading. In: *Pathology and Genetics of Tumors of Soft Tissue and Bone*. Lyon: IARCPress 2002, 12-18.
2. Fletcher CDM, Van den Berg E, Molenaar WM. Pleomorphic malignant fibrous histiocytoma / Undifferentiated high grade pleomorphic sarcoma. In: *Pathology and Genetics of Tumors of Soft Tissue and Bone*. Lyon: IARCPress, 2002, 120-122.
3. Lawrence W Jr, Donegan WL, Natarajan N, Mettlin C, Beart R, Winchester D. Adult soft tissue sarcomas. A pattern of care survey of the American College of Surgeons. *Ann Surg*. 1987; 205(4): 349-359.
4. Stacy GS, Yip D. Pleomorphic Sarcoma (Malignant Fibrous Histiocytoma) of Soft Tissue Imaging. Available: <http://emedicine.medscape.com/article/391453-overview#showall> 2015
5. Charest M, Hickeson M, Lisbona R, Novales-Diaz JA, Derbekyan V, Turcotte RE. FDG PET/CT imaging in primary osseous and soft tissue sarcomas: a retrospective review of 212 cases. *Eur J Nucl Med Mol Imaging* 2009; 36(12):1944-1951.
6. Roberge D, Hickeson M, Charest M, Turcotte RE. Initial McGill experience with fluorodeoxyglucose PET/CT staging of soft-tissue sarcoma, *Curr Oncol* 2010; 17(6):18-22
7. Coindre JM, Grading of Soft Tissue Sarcomas: Review and Update, *Arch Pathol Lab Med* 2006; 130(10): 1448-1453.
8. Piette AM, Fièvre prolongée. In: *Traité de Médecine Akos*. Paris : Elsevier Masson SAS 2009, 1-6.
9. Barrington S, Scarsbrook A. Indication for 18F-fluorodeoxyglucose (FDG) PET-CT. In: *Evidence-based indications for the use of PET-CT in the UK*, London: London: RCP, RCR 2013, 4-9,
10. Eary J, Conrad E. PET and Sarcoma. Available: <http://sarcomahelp.org/articles/PET-sarcoma.html> 2008
11. Chao C, Goldberg M. Surgical Treatment of Metastatic Pulmonary Soft-Tissue Sarcoma. *Oncology (Williston Park)* 2000; 14(6):835-841.
12. Fletcher CDM, Rydholm A, Singer S, Sundaram M, Coindre JM. Soft tissue tumors: Epidemiology, clinical features, histopathological typing and grading. In: *Pathology and Genetics of Tumors of Soft Tissue and Bone*. Lyon: IARCPress 2002, 12-18.

COMPLEX MANAGEMENT OF FRAILTY FRACTURES – CASE REPORT

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COMPLEX MANAGEMENT OF FRAILTY FRACTURES – CASE REPORT (Abstract):

Frailty fractures encompass pelvic ring fractures in the elderly – a condition more frequently encountered in women, usually with pre-existing osteoporosis. The incidence of these fractures is increasing, following the increase in average life expectancy. Most of the times, they are the result of a minor injury, of which the patient has little or no recollection; the clinical signs and symptoms, radiological changes and the degree of fracture stability vary greatly. As such, there is a wide range of therapeutic options and one of the most important decisions for the physician is choosing between surgery and conservative treatment. We present the case of an elderly female patient with multiple pelvic ring fractures, and a medical history of cardiovascular disease and osteoporosis; the orthopedic examination recommended conservative treatment which was carried out in the Geriatric Clinic of the “Dr. C.I. Parhon” Hospital. Following the complete physical examination and through geriatric evaluation, a complex therapeutic plan was devised, which included: recovery through physiotherapy, adequate nutrition, psychological support, treatment of the cardiovascular comorbidities and prevention of the consequences of extended bed rest. The treatment lasted for 60 days and the outcome was favorable: decreased bone frailty, mainly through preserving muscle tone and osteoarticular function, correction of malnutrition, improvement of depression and partial recovery of patient’s mobility; the results of our therapy made it possible for the patient to return home, where she leaves alone. She will need help with daily household chores, but she intends to continue the recovery therapy as means of increasing her quality of life and independence. **Keywords:** FRAILTY FRACTURES, SENIOR PATIENTS, RECOVERY THERAPY.

As the elderly segment of the population increases, so does the incidence of geriatric syndromes. Consequently, their impact on the medical services is more and more taxing, especially in the case of osteoarticular conditions that are associated with an increased risk of falling. Most frequently, patients with osteoporosis experi-

ence either hip or pelvic ring fractures (1). Pelvic ring fractures in the elderly are considered among frailty fractures (2) and are usually encountered in women over the age of 80. The gender-specific incidence has a different pattern according to age – from 0.54/0.38 % for patients aged 65-69 to 9.35/4.45 % for ages over 90; also, inci-

dence is higher for institutionalized or moderately disabled persons (3). Most often, pelvic ring fractures in the elderly are the consequence of a minor injury - either same-level fall at home or during the bed to chair transfer. Frequently, these fractures occur on an osteoporotic bone. Both pre-existing comorbidities and the clinical consequences of the fracture (significant pain, total immobilization, and dependency) darken the prognosis and require a thorough assessment prior to a therapeutic decision (3). There is no consensus regarding the orthopedic approach: surgery versus conservative treatment, both being associated with considerable risks (4, 5).

We present the case of an elderly female patient with multiple pelvic ring fractures, for whom a conservative treatment was recommended. She was subsequently transferred to the Geriatric Clinic of the "Dr. C.I. Parhon" Hospital for a concomitant cardiovascular complication. The comprehensive geriatric assessment and the therapeutic plan, which were managed by a multidisciplinary team, led to the satisfactory recovery of the patient and allowed her to return to her own home.

CASE REPORT

We present the case of an 80 year-old female patient, living in an urban house, who was admitted to the Orthopedics Department with multiple pelvic ring fractures (fracture of the anterior left acetabular wall without displacement, left ischiopubic fracture, sacral wing fracture) following a minor fall injury at home (fig. 1). She had a recent medical history of arterial hypertension (for which she was prescribed treatment with ACE inhibitors) and osteoporosis. During hospitalization, a routine cardiology consultation revealed an episode of

tachyarrhythmia (high frequency atrial fibrillation).



Fig. 1. The X-ray of the patient showing multiple pelvic ring fractures (fracture of the anterior left acetabular wall without displacement, left ischiopubic fracture, sacral wing fracture).

Due to the recently-diagnosed cardiovascular comorbidities and because the fractures were stable (no associated displacement), the orthopedic surgeon opted for conservative treatment, despite the fact that the fractures had occurred on a pathologic bone. The patient was transferred to the Geriatric Clinic for a thorough assessment prior to her discharge at home.

On admission, the patient had significant pain in the lumbosacral and pelvic areas, with partial bed immobilization (she could turn from one side to the other), mildly elevated blood pressure, psychomotor agitation, anxiety, and depression. Additional investigations did not identify other medical conditions except for the previously diagnosed arterial hypertension. Comprehensive geriatric assessment revealed: malnutrition (21/30 MNA score), complete dependency (assessed by ADL and IADL scales), moderate depression (assessed by the GDS scale), clinical frailty (assessed by the Freid scale) and the absence of cognitive disorders

(assessed by the MMSE scale). Both a physiotherapist and psychologist consulted the patient and participated in developing the management plan.

The therapeutic approach of the multi-disciplinary team focused on the following points: ensuring careful monitoring of the cardiovascular status, providing an appropriate diet for malnutrition, osteoporosis and heart disease, securing an immediate, suitable and continuous functional recovery, offering psychological counseling and qualified care to prevent complications of prolonged bed rest. Special attention was given to functional recovery, due to the complexity of the case. The treatment plan had to take into account the fact that fractures occurred on osteoporotic bones (6), that the orthopedic treatment consisted in relative bed rest (no cast immobilization and no stepping on the affected leg for 60 days), and that the patient wanted to return as soon as possible to her home, where she lived alone.

One of the most important roles was played by the physiotherapist, who had to attempt meeting the following aims:

a. decrease the pain that occurred mainly during the recovery sessions, but also while performing the smallest movements in the bed;

b. recovery, restoration and consolidation of the affected motor functions, correct and full recovery of force and joint mobility, muscle strength recovery in both upper and lower limbs (7);

c. restoration of stability, controlled movement and ability for body support when the patient began to walk using the frame, crutches and, at the end, the cane, with gradual and correct resumption of independent walking;

The procedures used to accomplish the

set goals were:

- electro acupuncture at acupoints in the painful area for instant pain relief, resulting in greater amplitude of the movements during passive, active-passive and active physiotherapy;

- sedative and relaxing therapeutic massage, which resulted in a significant decrease of pain. This procedure also had a positive mental effect on the patient, who's compliance during treatment increased; active involvement of the patient augmented the beneficial effects of physiotherapy, with excellent results despite osteoporosis and old age (which, usually, is a hindrance in the recovery dynamic);

- massage and reflexotherapy, which determined a growth in basal metabolic rate, stimulating respiratory and circulatory apparatus functions, improving sleep quality and the person's general state;

- breathing exercises and bilateral posterior chest massages in order to avoid bedsores and, more importantly, to prevent decubitus pneumonia.

Recovery treatment was gradually more complex. As the fracture consolidated, the pelvic member on the fracture's side was loaded progressively and the use of the thoracic limb was encouraged (8). The patient's relative bed rest lasted for 3 weeks as per orthopedic recommendations for stable fractures. At the beginning of the third week, the patient sat up at the side of the bed, and, by the fourth week, she managed standing up and performing short movements (without pressuring the affected limb), with the help of an attendant and the walking frame, which enables good control of body weight distribution and center of gravity adjustment. At the same time, improvement of the nutritional status, decreased anxiety and depression, and

efficient control of blood pressure values were achieved. 60 days after the injury, the patient was discharged with a relative dependency status (walking by cane) and need of in-home help.

DISCUSSIONS

Given the improved life expectancy, the rising incidence of osteoporosis and frailty inevitably led to an increase in pelvic ring fracture rates, which are considered frailty fractures. They typically involve elderly females (over 80 years old), are diagnosed after a minor injury (sometimes the patient does not remember when the trauma occurred) and have disastrous consequences for the patient and their caregivers: prolonged bed rest with severe deterioration of the muscles, joints and bones associated with increased dependency level (in some cases, institutionalization is needed due to the inability to return home). This represents an important health care burden both from a social and an economic point of view (9).

Conservative treatment, which is preferred in the stable fractures of patients with clinical frailty, cardiovascular comorbidities, accelerated osteoporosis and malnutrition is associated with a long period of immobilization and bed rest, and, inevitably, all its complications. The complex treatment (physiotherapy, psychotherapy, proper nutrition and continuous medical monitoring), performed in a specialized medical unit, is the main step towards ther-

apeutic success, allowing complex patient recovery and restoring quality of life.

Because the Romanian health care system lacks specialized centers for complex recovery of the elderly, this program was implemented at the Geriatric Clinic, where constant supervision and medical care, nutritional counseling, psychological assistance and a thorough physiotherapy schedule, together with patient's compliance and her wish to return home, led to the recovery of walking ability, with a significant decrease of the dependency level, and allowed for a quality of life status similar to that prior to the fracture.

CONCLUSION

Pelvic ring fractures in the elderly are considered among frailty fractures because they are usually encountered in women over the age of 80. There is no consensus regarding the orthopedic approach: surgery versus conservative treatment, both being associated with considerable risks. The particularities of old and very old age (frailty and osteoporosis, the tendency to malnutrition, depression and cognitive impairment, the concomitant chronic diseases) need a comprehensive geriatric assessment performed by a multidisciplinary team who is well trained to devise a complex recovery plan, performed in a specialized clinic and which would allow the patient to return, as much as possible, to the way of life that existed prior to fracture.

REFERENCES

1. Dodge G, Brison R. Low-impact pelvic fractures in the emergency department. *CJEM*. 2010;12(6):509–513.
2. Kates SL, Bukata SV, DiGiovanni BF, Friedman SM, Hoyen H, Kates A, Kates SL, Mears SC, Mendelson DA, Serna FH, Jr., Sieber FE and Tyler WK. A guide to improving the care of patients with fragility fractures. *Geriatric orthopaedic surgery and rehabilitation* 2011; 2: 5-37.

3. Benzinger P, Becker C, Kerse N, et al. Pelvic fracture rates in community-living people with and without disability and in residents of nursing homes. *J Am Med Dir Assoc*; 2013 Sep;14(9):673-8.
4. Rommens PM, Hofmann A. Comprehensive classification of fragility fractures of the pelvic ring. Recommendations for surgical treatment. *Injury*. 2013;44(12):1733–1744. doi: 10.1016/j.injury.2013.06.023
5. Arduini M, Saturnino L, Piperno A, Iundusi R, Tarantino U. Fragility fractures of the pelvis: treatment and preliminary results. *Aging ClinExp Res*, 2015; 27 Suppl 1:61-7.
6. Furey AJ, O’ Toole RV, Nascone JW, et al: Classification of pelvic fractures: analysis of inter- and intraobserver variability using the Young-Burgess and Tile classification systems. *Orthopedics*. 2009 Jun;32(6):401. doi: 10.3928/01477447-20090511-05.
7. Eckroth-Bernard K, Davis JW: Management of pelvic fractures. *Curr Opin Crit Care*. 2010 Dec;16(6):582-6. doi: 10.1097/MCC.0b013e3283402869.
8. Sbenghe T: *Kinetologieprofilactică, terapeutică și de recuperare*, Ed. Medicală, 1987
9. Andrich S, Haastert B, Neuhaus E, et al. Epidemiology of Pelvic Fractures in Germany: Considerably High Incidence Rates among Older People. *PLoS One*. 2015; 10(9): e0139078.

NEWS

GINGIVAL, PLASMA AND SALIVARY LEVELS OF MELATONIN IN PERIODONTALLY HEALTHY INDIVIDUALS AND CHRONIC PERIODONTITIS PATIENTS: A PILOT STUDY

Melatonin (N-Acetyl-5-hydroxytryptamine) is an indole amine hormone synthesized predominantly by the pineal gland. Extrapineal synthesis and release of melatonin can occur from organs like ovaries, retina, gastrointestinal tract and also from cells such as human lymphocytes. Melatonin regulates circadian rhythm and the biological clock in the human body. In addition, it performs numerous other functions like immunomodulation and osteopromotion. Melatonin has potent antioxidant action against reactive oxygen species and can mitigate deleterious effects of free radical damage in the human body. It is well known that chronic periodontitis is an inflammatory condition of the tooth supporting structures with a microbial aetiology, in which dysregulated immune response and a jeopardized oxidant - anti oxidant balance play a major role in causing tissue destruction and tooth loss. Previous studies have shown increased oxidative stress markers and lowered antioxidant status in periodontal disease compared to health. In this context, the presence of melatonin as an antioxidant in saliva and plasma and its association with the pathogenesis of chronic periodontitis has been researched. It has been hypothesized that salivary melatonin can act as a potent local antioxidant boosting the defence mechanism against periodontal pathology. Till date no study has investigated, melatonin levels in the gingival tissue. This study demonstrates the presence of melatonin in gingival tissue. Furthermore, melatonin levels are lowered in gingival tissues of chronic periodontitis patients (Balaji TM, Vasanthi HV, Rao SR, et al. *Gingival, Plasma and Salivary Levels of Melatonin in Periodontally Healthy Individuals and Chronic Periodontitis Patients: A Pilot Study*. *Journal of Clinical and Diagnostic Research*. 2015, Vol-9(3): 23-25).

Doina Butcovan

SEVERE AMIODARONE-INDUCED BRADICARDIA CONCEALES SICK SINUS SYNDROME: CASE REPORT

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SEVERE AMIODARONE-INDUCED BRADICARDIA CONCEALS SICK SINUS SYNDROME: CASE REPORT (Abstract). Sinus node dysfunction is one of the most common arrhythmias in elderly patients; it is usually associated with intermittent and variable symptoms, thus making it difficult to diagnose. We present the case of an elderly female patient with a personal history of atrial fibrillation treated for the last three years with amiodarone; she was admitted to the Geriatric Clinic for non-specific symptoms with onset two months previously for which she had already sought care in different medical services. Clinical examination showed severe bradycardia; ECG and Holter ECG on admission confirmed severe bradycardia, with a heart rate between 29 and 50 beats/min (bpm). Given her long-term treatment with amiodarone we looked for and found hyperthyroidism; the endocrine examination led to the diagnosis of mixed type Amiodarone-induced thyrotoxicosis and initiation of corticosteroid and antithyroid treatment. The evolution of cardiac arrhythmia was monitored with the help of several Holter ECGs performed after amiodarone washout and return to the euthyroid state, which revealed a tachycardia-bradycardia syndrome initially masked by the side effects of the unsupervised therapy with amiodarone, and properly treated by the implantation of a pacemaker. **Keywords:** SINUS NODE DYSFUNCTION, TACHYCARDIA-BRADYCARDIA SYNDROME, HYPERTHYROIDISM, AMIODARONE.

There are several normal age-related changes of the cardiovascular system, one of the most important affecting the conduction system with increasing fibrosis around the sinus node and loss of specialized cells and fibers. These changes, associated with a decreased response to sympathetic agonists would lead to a decreased peak heart rate and a significant decrease in heart rate variability, resulting in bradycardia, which may be further exacerbated by medication and ischemia (1). Older patients often have

delayed conduction in the atrio-ventricular (AV) node, which would generate different types of AV blocks, that are usually without symptoms and could progress to complete AV block (2).

These significant changes in the anatomy and function of the conduction system of the aging heart explain the increased frequency of sick sinus syndrome, a group of arrhythmias presumably caused by a malfunction of the sinus node, including sinus arrest, sinus node exit block, sinus bradycardia and tach-

ycardia-bradycardia syndrome.

The manifestations of this disease are diverse, intermittent and often difficult to surprise on casual ECG (3), reason for which the epidemiological data are limited and the incidence of this disease in the general population remains unclear (4, 5). Syncope and/or fatigue are the cardinal symptoms in the bradycardia forms, and palpitations in the tachycardia forms, but it is not uncommon (especially in the elderly) that symptoms to be limited to dyspnea, reduced exercise capacity or mild cognitive impairment (sometimes interpreted as the onset of dementia syndrome) (6). In this context, Holter ECG monitoring remains the investigation of choice, identifying not only the accurate diagnosis but also the right moment for the implantation of pacemaker.

When confronted with fast atrial fibrillation, controlling the ventricular rate should be of the outmost importance, and the favorite antiarrhythmic drug is amiodarone (5). This trend led to a massive use of this drug in elderly patients, which was accompanied by a constantly increasing number of unwanted side effects, most frequently occurring at thyroid level. The lack of periodic and systematic control can be accompanied by serious clinical situations, such as severe bradycardia, that can be masked by nonspecific symptoms and thus easily overlooked and undertreated.

CASE PRESENTATION

We present the case of an 86 year old female patient admitted to the Geriatric Clinic for several non-specific symptoms, such as: dry and irritative cough, vertigo and generalized itching with nocturnal exacerbation. The onset of these symptoms was insidious about two months prior to this hospitalization, the patient having already sought care in different medical services. On ad-

mission she presented: facial erythema, scratching lesions on the posterior chest wall, rhythmic heart rate of 46 beats/min, and bilateral upper limb resting tremor. Her medical history revealed an episode of fast atrial fibrillation that had occurred three years earlier and was successfully treated with amiodarone, although the biochemical thyroid function tests indicated at that moment subclinical hyperthyroidism, with TSH concentration at the lower limit of normal range but with fT4 and fT3 concentrations within the normal range.

Our diagnosis was severe bradycardia, most probably due to thyroid dysfunction secondary to the long-term, unsupervised treatment with amiodarone. The biochemical tests revealed very low serum levels of TSH hormone (TSH = 0.01 pmol/l), high levels of fT4 (fT4 = 30.8 μ UI/ L), and normal levels of fT3. ECG on admission confirmed sinus bradycardia (Figure 1); however, the 24-hour ECG monitoring showed sinus bradycardia alternating with idioventricular rhythm; the average heart rate was 35 bpm, range 29 to 50 bpm; the absence of any cardio-cerebral symptoms allowed the postponing of pacemaker implantation.

The endocrine examination confirmed the diagnosis of mixed type amiodarone-induced thyrotoxicosis, and a treatment with corticosteroids and antithyroid drugs was initiated.

The clinical course was favorable, as the patient reported the disappearance of dry cough and prurigo 48 hours after the discontinuation of amiodarone and the heart rate slowly ascended (up to 50 bpm). A 24-hour ECG monitoring 1 month after the discontinuation of amiodarone treatment showed an average heart rate of 45 bpm, range 39 to 100 bpm, which led us to suspect an underlying, unsuspected bradycardia-tachycardia

syndrome. A third 24-hour ECG monitoring was done after 6 weeks of thyroid treatment, when thyroid function had already returned to normal; hence, we considered that this one would reflect the correct cardiac status

of our patient'. The result was very much the same as the previous one, thus confirming the diagnosis of bradycardia-tachycardia syndrome, for which a pacemaker was implanted.

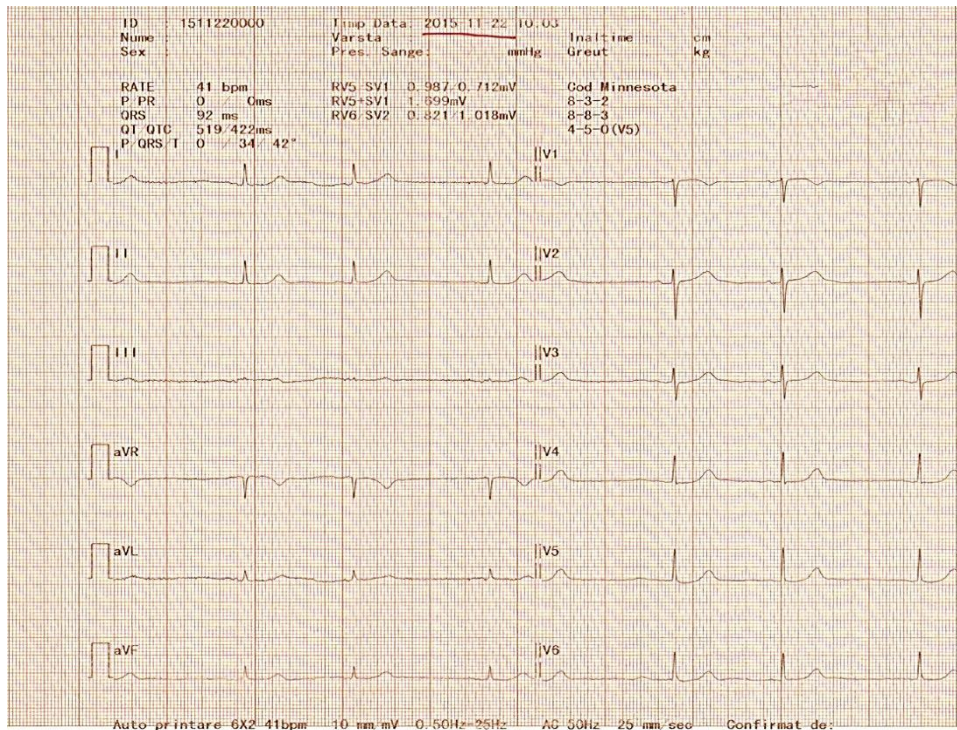


Fig. 1. ECG aspect on admission

DISCUSSION

The normal aging process of the heart includes fibrosis and loss of specialized cells and fibers of the conductive system, which would favor an increased incidence of sick node dysfunction. This condition can take many forms, including bradycardia-tachycardia syndrome. The diagnosis of this form can be difficult, as random ECG is more often normal. The bradycardia episodes are usually without any symptoms; the tachycardia episodes are associated with palpitations and the patient is wrongly diagnosed with only tach-

yarrhythmia and would receive an anti-arrhythmic treatment, usually amiodarone. This drug should be carefully monitored, not only for the well-known side-effects, but also for the negative effect on the bradycardia episodes.

Numerous studies (7, 8, 9) show that low doses of amiodarone can be used safely in patients with fast arrhythmias and normal systolic function. However, the length of the treatment with amiodarone proved to be an independent risk factor in the prediction of adverse effects (4), mainly changes at the cardiovascular (im-

portant bradycardia), thyroid (hyper- and hypothyroidism), pulmonary and hepatic level (9, 10).

Still, the absence of cardiac symptoms (syncope, fatigue) and hyperthyroidism led to a delay in the diagnosis. This case also presented some of the less known side effects of amiodarone, that include nervous system disorders (paresthesia, pain and muscle weakness, tremor, walking and moving disorders, headache, vertigo), gastrointestinal disorders (nausea, taste alteration, constipation, lack of appetite) or skin disorders (itching, petechiae) (8) that can be easily interpreted as a concomitant chronic or aging-related disease.

CONCLUSIONS

Our case emphasizes the need for sustained monitoring of treatment with amiodarone; the multiple side effects (cardiovascular and thyroid) associated with the progression to a higher-degree AV block led to severe bradycardia, fortunately without cerebral and cardiac consequences.

The therapeutic decision, especially in tachycardia-bradycardia syndrome should be carefully considered; the patients that require antiarrhythmic therapy should be rigorously monitored, mainly those treated with amiodarone, and the 24-hour ECG monitoring should be the safest way for identifying the excessive secondary “bradycardization”.

REFERENCES

1. Hazzard's *Geriatric Medicine and Gerontology*, Sixth Edition, Part B, Cardiology, Cardiac Arrhythmias, 951-967.
2. Williams BA, Chang A: *Geriatrics. Current Diagnosis & Treatment*, Second Edition, McGraw Hill Education, 2014, ch. 29, 190-201.
3. Adan V, Crown LA, Diagnosis and Treatment of Sick Sinus Syndrome. *Am Fam Physician*. 2003 15;67(8):1725-1732.
4. Jensen PN, Gronroos NN, Chen LY, Folsom AR, DeFilippi C, Heckbert SR, Alonso A, Incidence of and Risk Factors for Sick Sinus Syndrome in the General Population. *J Am Coll Cardiol*. 2014 12; 64(6): 531–538.
5. Wann LS, Curtis AB, Ellenbogen KA, et al. Management of patients with atrial fibrillation (compilation of 2006 ACCF/AHA/ESC and 2011 ACCF/AHA/HRS recommendations): a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines. *Circulation*. 2013;127(18):1916–1926.
6. Rodriguez RD, Schocken DD. Update on sick sinus syndrome, a cardiac disorder of aging. *Geriatrics*. 1990;45:26-30.
7. Karamichalakis N, Letsas KP, Vlachos K, Georgopoulos S, Bakalakos A, Efremidis M, Sideris A. Managing atrial fibrillation in the very elderly patient: challenges and solutions. *Vasc Health Risk Manag*. 2015 Oct 27;11:555-562.
8. Kim HL, Seo JB, Chung WY, Kim SH, Kim MA, Zo JH. The incidence and predictors of overall adverse effects caused by low dose amiodarone in real-world clinical practice. *Korean J Intern Med*. 2014 Sep;29(5):588-596.
9. Lafuente-Lafuente C, Alvarez JC, Leenhardt A, Mouly S, Extramiana F, Caulin C, Funck-Brentano C, Bergmann JF. Amiodarone concentrations in plasma and fat tissue during chronic treatment and related toxicity. *Br J Clin Pharmacol*. 2009; 67(5): 511–519.
10. Doylend JF, Kwok M. Benefits and Risks of Long-term Amiodarone Therapy for Persistent Atrial Fibrillation: A Meta-analysis. *Mayo Clin Proc*. 2009; 84(3): 234–242.

ATYPICAL FEMORAL FRACTURES AFTER LONG-TERM BISPHOSPHONATES THERAPY: CASE REPORT

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ATYPICAL FEMORAL FRACTURES AFTER LONG-TERM BISPHOSPHONATES THERAPY: CASE REPORT. (Abstract). We present a 77 years-old woman with no history of trauma, or associated with low-energy trauma, admitted to our clinic after three weeks of a left femoral fracture threat in Orthopedic Clinic. The patient was in treatment with bisphosphonates over 10 years for osteoporosis. **Discussion and conclusions:** The causal relationship between prolonged bisphosphonate use and the occurrence of atypical femoral fractures (AFF) has not yet been established. For the patient at high risk of fracture, it may be beneficial to continue bisphosphonate treatment beyond five years. The absolute risk of atypical femoral fractures is low (about 100 cases per 100,000 person-years among long-term users). For most people with osteoporosis, the proven fragility-fracture risk-reduction benefits of bisphosphonates outweigh the risks of AFF. **Keywords:** ATYPICAL FEMORAL FRACTURE; BISPHOSPHONATES; OSTEOPOROSIS.

Bisphosphonates are a class of widely prescribed drugs that are proven to be effective in reducing common bone fractures in people with osteoporosis and those at a high risk of fractures. Concern among doctors and patients has arisen following recent media reports that cite a possible association between unusual and unexpected (atypical) fractures of the upper thigh bone and bisphosphonate use. Recent published studies (1) examining this potential association are conflicting regarding the existence and strength of this association. Bisphosphonate-related proximal femoral fractures are an example

of insufficiency fractures, although the direct causative link remains somewhat controversial (1). According to a task force report from the American Society for Bone and Mineral Research (ASBMR), this relationship has not yet been shown to be causal. All current evidence indicates that atypical femoral fractures (AFF) represent a rare subset of subtrochanteric and femoral shaft fractures (2). Femoral shaft fractures had an incidence of 2.5 to 9.9 per 100,000 person-years, but 75% occurred with high-energy trauma and the majority were comminuted and had a spiral configuration (3). A review of radiographs by

Schilcher et al. (4) found an incidence of 55 atypical fractures per 100,000 person-years among bisphosphonate users compared with 0.9 per 100,000 person-years among those with no bisphosphonate use. Characteristics of AFF are: a transverse, noncomminuted fracture at the subtrochanteric or femoral shaft region with a medial cortical spike at the fracture area. Other features include prodromal pain and generalized thickening of the femoral cortices on radiographs. For the clear criteria to define atypical femoral fractures, the ASBMR task force established major and minor features for both complete and incomplete atypical fractures of the femur (2). All of the major features should be present to designate a fracture as atypical: No history of trauma, or associated with low-energy trauma; Fracture located anywhere from distal to the lesser trochanter to proximal to the supracondylar area; Transverse or short oblique fracture configuration; Noncomminuted fracture; Medial spike in complete fractures, incomplete fractures involve only the lateral cortex. The minor features have also been described in association with atypical femoral fractures, but they are not required for diagnosis. Localized periosteal thickening of the lateral cortex; Generalized thickening of the femoral cortices; Prodromal symptoms; May be associated with bilateral fractures or symptoms; Evidence of delayed fracture-healing; Comorbid conditions or the use of some medications.

CASE REPORT

A 77 years-old woman, weight 62 kg and height 1.58 was admitted to Endocrinology clinic after three weeks of a left femur fracture in mid-thigh, threatened in

Orthopedic Clinic (fig. 1).



Fig. 1. The patient after orthopedic surgery

Orthopedic Surgery underwent the closed reduction and internal fixation with intra medullary hip screw (fig.2 b). The patient was in our clinical observation since 1983 for hypothyroidism after thyroidectomy in treatment with 100 mcg LT4. In 2002 the patient was diagnosed with osteoporosis following the DXA examination. The bone mineral density (BMD), measured by dual-energy X-ray absorptiometry (DXA) showed T-score at total lumbar spine: -4.0, total hip: -2.1, and left femur neck: -3.0. 25-OHD3 level varied between 17.69 ng/mL and 5.54 ng/mL (normal >30 ng/mL). Thyroid Stimulating Hormone, thyroxin and triiodothyronine level was normal. Has been established treatment with risedronicum acidum 35 mg/week (Actonel), Alfacalcidol 1 mcg/day and calcium 1000 mg/day. Annual DXA-BMD shows a slight increase the density of the bone; patient medical treatment has been changed in zoledronicum acidum and then on alendronicum acidum.

In 2013 there is a decrease of BMD DXA, but our patient requires the continuation of the therapy with a drug with large intervals administration. Ibandronic acid has been recommended. After surgical orthopedic treatment was discussed with the patient, the risks and benefits of each option. The new antiepileptic treatment was strontium ranelate in association with Alfacalcidol and calcium. An association

between bisphosphonate long-term use and the occurrence of AFF has been suggested. This diagnosis has been supported and by the x-ray. The radiographic features of an atypical femoral fracture: femoral shaft region (fig. 2a), transverse fracture configurations, absence of comminution, a medial spike, localized periosteal thickening of the lateral cortex, and generalized thickening of the femoral cortices.



Fig. 2 a. transverse fracture configurations, absence of comminution, a medial spike, localized periosteal thickening of the lateral cortex, and generalized thickening of the femoral cortices. b. Post-operative radiograph after surgical fixation. c. Post-operative radiograph after 3 month.

DISCUSSION

AFF have been associated with various factors, including: bilateral fractures, prodromal pain, the use of glucocorticoids and proton pump inhibitors, delayed fracture-healing, presence of rheumatoid arthritis or diabetes mellitus as well as vitamin-D3 deficiencies (5, 6). A post hoc analysis of data from three randomized trials of bisphosphonates for postmenopausal osteoporosis identified twelve subtrochanteric or

diaphyseal femoral fractures in ten of the 14,195 study participants, yielding a rate of 23 per 100,000 person-years. Reanalysis of the data in three major randomized controlled trials showed no statistically significant increase in the risk of subtrochanteric femoral fracture in patients treated with bisphosphonates for as long as ten years (2). Given the mixture of results and the different methodologies used in these studies, it is difficult to demonstrate consistent

cy of this association. Studies (7) have shown an increased risk of atypical femoral fractures in patients taking bisphosphonates for five or more years, suggesting a dose-response relationship. The ASBMR task force reported an incidence of 2 per 100,000 cases per year after two years of bisphosphonate use, increasing to 78 per 100,000 cases per year after eight years of use. Management of patients with AFF: Stop bisphosphonates. Consider an anabolic agent such as teriparatide. Calcium and vitamin-D supplementation (6). Identify any underlying metabolic bone diseases by laboratory investigations such as serum calcium, phosphate, 25-hydroxyvitamin D, intact parathyroid hormone, thyroid-stimulating hormone, bone turnover markers, and 24-hour urine calcium. Intramedullary nailing is the preferred method of fixation. Assess the contralateral femur. If the patient has an incomplete fracture, a period of conservative therapy may be considered. Five of six patients had progression of the lesion to complete fracture at a mean of ten months. Management of patients with prolonged bisphosphonate therapy: If a patient sustains an atypical femoral fracture, bisphosphonates must be stopped. Once administered, bisphosphonates accumulate in the bone and continue to be released for months or years after treatment

is discontinued. Regarding over suppression of bone turnover with prolonged bisphosphonate therapy, it is recommended that osteoporosis treatment with bisphosphonates be stopped after a period of five years to provide patients a so-called “drug holiday”. Park-Wyllie et al. performed a nested case-control study to explore the association between bisphosphonate use and femoral fractures, and they reported that bisphosphonates treatment of more than five years was associated with an increased risk of atypical subtrochanteric or femoral shaft fractures (7). Therefore, it may be appropriate to consider a drug holiday in patients with a cumulative duration of bisphosphonate treatment of more than five years. The absolute risk of atypical femoral fractures is low (3.2 to 50 cases per 100,000 person-years among short-term bisphosphonate users and about 100 cases per 100,000 person-years among long-term users). Consequently, for most people with osteoporosis, the proven fragility-fracture risk-reduction benefits of bisphosphonates outweigh the risks of AFF (8).

CONCLUSIONS

Patient confirms the risk of atypical femoral fractures in prolonged bisphosphonate therapy, but let us not forgets benefits of bisphosphonates.

REFERENCES

1. Yoon RS, Hwang JS, Beebe KS. Long-term bisphosphonate usage and subtrochanteric insufficiency fractures: a cause for concern? *J Bone Joint Surg Br.* 2011; 93 (10): 1289-1295.
2. Shane E, Burr D, Ebeling PR, et al; Atypical subtrochanteric and diaphyseal femoral fractures: report of a task force of the American Society for Bone and Mineral Research. *J Bone Miner Res.* 2010; 25(11):2267-2294.
3. Wang Z, Bhattacharyya T. Trends in incidence of subtrochanteric fragility fractures and bisphosphonate use among the US elderly, 1996-2007. *J Bone Miner Res.* 2011; 26(3):553-560.

4. Schilcher J, Michaelsson K, Aspenberg P. Bisphosphonate use and atypical Fractures of the femoral shaft.. *N Engl J Med*. 2011; 365(16):1551-1559.
5. Galesanu C, Florescu A, Ungureanu MC et al. Vitamin D status in patients admitted in a clinic of Endocrinology. *Rev. Med. Chir. Soc. Med. Nat., Iași*. 2014; 118, (2):327-332
6. Galesanu C, Mocanu V. Vitamin D Deficiency and the Clinical Consequences. *Rev Med Chir Soc Med Nat Iasi*. 2015; 119(2):310-318.
7. Park-Wyllie LY, Mamdani MM, Juurlink DN, et al.. Bisphosphonate use and the risk of subtrochanteric femoral shaft fractures in older women. *JAMA*. 2011 23; 305(8):783-789.
8. Blood T, Feller R. J, Cohen E, et al. Atypical Fractures of the Femur: Evaluation and Treatment. *JBJS Reviews*, 2015; 3 (3): e1 DOI: 10.2106/JBJS.RVW.N.00062.

NEWS

SHED - BASIC STRUCTURE FOR STEM CELL RESEARCH

Stem cells are defined as clonogenic cells capable of both self-renewal and multi lineage differentiation. Stem cells have been differentiated from variety of body parts and have shown great promise in the management of variety of diseases in medicine. Recently, stem cells have been isolated and grown from: Human permanent dental pulp tissue stem cells (DPSCs), Stem cells from human exfoliated deciduous teeth (SHEDs), and Periodontal ligament apical papilla of immature teeth (PDLSCs). If we have in hand population of stem cells that reproducibly reform bone, cementum, dentin, and perhaps even periodontal ligament; it is possible to envision complete restoration of the hard tissues in the oral cavity using the patient's own cells, thereby avoiding issues of histocompatibility. This would be a more biological approach rather mere mechanical one. It has been reported that the stem cells obtained from the above said sources can generate dentin like tissue both in vitro and in vivo studies in animals. In addition transplanted skeletal or dental stem cells have the potential to "rephrase" repair craniofacial defects and repair/regenerate teeth. This review study showed that stem cells from human exfoliated deciduous teeth are distinct from dental pulp stem cells by virtue of their proliferation rate, increased cell population doublings and osteoinductive capacity in vivo. It also demonstrated that human exfoliated deciduous teeth stem cells may not be a single-cell type, may well be a heterogenous population of cells from the pulp (Kashyap R. SHED - Basic Structure for Stem Cell Research. *Journal of Clinical and Diagnostic Research*. 2015 Mar, Vol-9(3): 7-9).

Doina Butcovan

THE PATIENT-DOCTOR-PSYCHOLOGIST TRIANGLE IN A CASE OF SEVERE IMUNOSUPPRESSION IN THE HIV INFECTION

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THE PATIENT-DOCTOR-PSYCHOLOGIST TRIANGLE IN A CASE OF SEVERE IMUNOSUPPRESSION IN THE HIV INFECTION (Abstract). In the last two years the Romanian adult population infected with the human immunodeficiency virus (HIV) has increased due to sexual transmission, both heterosexual and homosexual. The case presented is that of a 33 year-old man, admitted to the Infectious Diseases Hospital in Iasi with acute respiratory failure and a confirmation of Kaposi's sarcoma. Tests later proved positive for HIV, the patient being included in the stage AIDS C3 (acute immunodeficiency syndrome). The respiratory failure was suspected to be caused by *Pneumocystis carinii* and cotrimoxazol therapy, oxygen therapy and anti-retroviral therapy were established. He was also referred to the oncology hospital for treatment of Kaposi's sarcoma. The patient's adherence to therapy was influenced by a strong doctor-patient relationship, as well as by psychological counseling and support. Creating a functional doctor-patient-psychologist team is key throughout the HIV-positive patient's existence, for supporting long term adherence to therapy and acceptance of the diagnosis. This case highlights the need for a strong psycho-social compartment in every medical center that deals with HIV-infected individuals. **Keywords:** HIV/AIDS, ANTIRETROVIRAL THERAPY, PSYCHOLOGICAL COUNSELING, DOCTOR-PATIENT RELATIONSHIP.

The HIV/AIDS pandemic has reached 36.9 million people globally living with HIV. At the end of 2014, two million people became newly infected with HIV, and 1.2 million people died from AIDS-related illnesses (1).

In Romania, the main characteristic of the HIV/AIDS population is the development of the “pediatric cohort”, represented by HIV positive youth infected as children in the 1990's (2). This group is currently

being equaled in numbers by newly infected cases. The most common means of transmission for these new cases are unprotected sexual intercourse, both heterosexual and homosexual, as well as use of injected drugs (3).

In the North-Eastern part of Romania, in October 2015 there were over 1400 people living with HIV/AIDS, this year being the first in which the number of newly diagnosed adults has equaled that of the

“pediatric cohort”. The infection is transmitted mostly by heterosexual and homosexual unprotected intercourse and less through intravenous administration.

CASE PRESENTATION

We present the case of a male patient, born in 1980. This patient was transferred to the HIV/AIDS Regional Center by a local clinic, in August 2013. The reasons for hospitalization were: weight loss, fatigue, impaired general condition, acute respiratory failure, perioronasal cyanosis and of the extremities, with preserved consciousness. At admission, the weight was 53 kg, height 163 cm and 76% partial oxygen pressure.

From the patient’s statements, his weight decreased progressively, 12 kg in 4 months. For the last 10 months, he developed lenticular brown spots on the skin, disseminated on the trunk, abdomen, and the upper right eyelid. There were no significant pathological aspects in the personal and family history. The biological samples from the County Hospital were near normal, except for hemoglobin 10.8 g/l, the appearance of “mat glass” of the chest X-ray, and also the positive result for HIV 1 and 2 ELISA test. Upon more in depth interrogations about the patient’s personal history, he identifies the means of getting infected with HIV as unprotected homosexual intercourse.

Viro-immunologic tests show a profound immunosuppression, with CD4 2 cells/mm³ (fig. 1) and a viral load (VL) of 518000 copies/ml (fig. 2). The dermatologic examination established the clinical diagnosis of Kaposi sarcoma, confirmed by skin biopsy. Serology for cytomegalovirus (CMV), toxoplasma, hepatitis B and C were negative, with also a negative VDRL. The major

suspicion in the context of pulmonary failure was pulmonary pneumocystis, and therapy was initiated with cotrimoxazole 8 tablets / day, intermittent oxygen, decreasing cortisol dose. Antiretroviral therapy immediately instituted was achieved with co-formulations of lopinavir + ritonavir and lamivudine + zidovudine and enfuvirtide (T20 Fuzeon). The pulmonary phenomena remitted extremely slowly, with the possibility of transfer within 30 days in the oncology ward of the county hospital, where specific therapy for Kaposi's sarcoma was initiated.

The patient, being a university graduate, showed a high level of intelligence and seemed to understand his situation. The infectious diseases doctor managed to create a relationship with this patient based on mutual trust, which encouraged him to own his diagnosis, expressing the desire to overcome this obstacle.

Throughout the hospitalization the patient benefited from psychological counseling and support after diagnosis disclosure. This added the psychologist to his support network, alongside the infectious diseases doctor. Counseling sessions consisted in semi-structured interviews about the patient’s personal and medical history, as well as free discussions regarding topics of interest for him – family, work-place, intimate relationships, social stigma. The main objective of was to explore fears and emotions linked to the HIV diagnosis, assisting the patient in dealing with them in a healthy and productive manner. The result of counseling and support were seen in a good compliance and adherence to antiretroviral (ARV) therapy, while resuming his daily routines and activities.

Three months after the first episode, the respiratory failure was resolved. The CD4

cell count increased to 92 cells/mm³ (fig. 1), and the viral load decreased over 100 times, VL 5530 copies/ml (fig. 2). Cotrimoxazole prophylaxis was maintained, reducing the dose to 2 tablets every 2 days, with continued ARV treatment and specific cancer therapy.

In May 2014 the viral load remained

high viral and CD4 increased slightly.

However, in October 2014 VL grew to 12,800 copies/ml (fig. 2) and CD4 cell count dropped to 65 cells/mm³ (fig. 1). The patient admitted that, after a period of good general condition, and after discontinuing therapy with enfuvirtide, he began to neglect antiretroviral therapy up to 3-4 days a week.

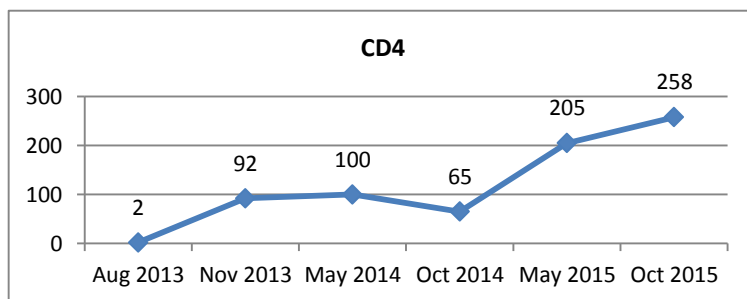


Fig. 1. CD4 cell count

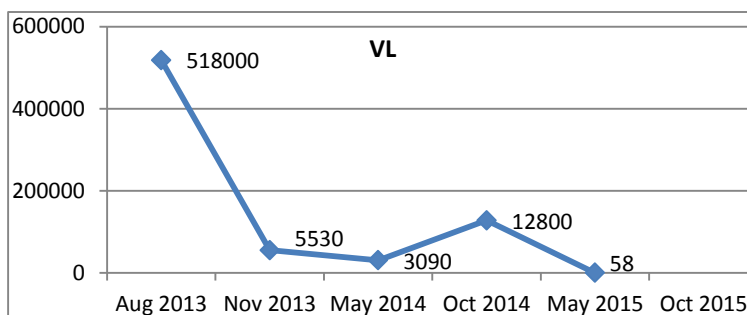


Fig. 2. Viral load

Daily psychological sessions were resumed, as well as repeated discussions with the infectious diseases doctor. These aimed to explore the patient's reasons for non-adherence and help him identify ways to prevent this in the future. The main objective was to increase long term adherence and compliance to ARV therapy. On discharge the patient seemed willing to maintain his adherence to therapy.

In the month of May 2015, biological

evaluations showed a CD4 count of 205 cells/mm³ (fig. 1), and VL a little over the detection limit - 58 copies/ml (fig. 1), with a weight gain of 8 kg. The oncology therapy with interferon solved the Kaposi lesion on the upper left eyelid and faded the other elements on the skin. The prophylaxis for pneumocystis with Cotrimoxazole was suppressed because CD4 cell count rose above 200 cells / mm³.

On the 29th of October 2015 the patient

had 78 kg, with a plus of 25 kg compared to the time of his first presentation. The Kaposi sarcoma faded, his viral load was undetectable, and CD4 was 258 copies / mmc.

DISCUSSIONS

The case presented is that of an intellectual patient with very severe immunosuppression caused by HIV infection. He had the chance to overcome a complex, extremely aggressive and life-threatening pathology, with acute respiratory failure and disseminated Kaposi sarcoma.

Initially, his results were good, due to the possibility of a smooth communication and understanding of diagnosis and both therapy benefits and risks of non-adherence.

The patient received psychological support throughout the hospitalization. Counseling sessions focused on internalizing information regarding the diagnosis, and especially on building a positive self-image, strengthening self-respect and shaping positive future projections.

The therapy was based on the very probable etiology of respiratory failure – that of pneumocystosis, as well as on the Kaposi's sarcoma. This specific therapy, alongside antiretroviral therapy, encouraged an increase of the immune competence over time, successfully managing the case (4,5).

However, therapeutic indications were apparently not enough. The patient's high coefficient of intelligence and education were a baseline in establishing a productive patient-doctor relationship, but were not sufficient in providing long-term support for adherence. Daily clinical practice in the

HIV/AIDS Regional Center in Iasi proved that, in a chronic disease such as HIV/AIDS, with lifelong daily therapy, psychological sessions are essential in offering support and helping patients find resources to cope (6,7,8).

CONCLUSIONS

Given the complexity of hospitalized cases, the management of an HIV-positive patient should be a team effort. In our study, we highlighted the fact that trust in the infectious diseases doctor, as well as confidence in one's strength to overcome the burden of the diagnosis are as important as ARV therapy or treating opportunistic infections.

The psychologist's intake is essential throughout the life of HIV infected patients; repeated counseling sessions are important for optimal adherence and compliance to therapy, in order to achieve viro-immunologic success and social integration. The need to adapt daily life and routines to new diagnosis requires its total acceptance; the support that people diagnosed with HIV receive facilitates this process of adaptation.

Therefore the development of a psychosocial department with trained professionals in the pathology of HIV/AIDS is more than necessary in managing the success of any case of HIV infection. The triangle formed by the doctor, the patient and the psychologist, working as a team toward a common goal, is the key to a successful management of the disease and should be a model included in every center that treats HIV pathology.

REFERENCES

1. UNAIDS 2014 Global Statistics. Onlin at:
http://www.unaids.org/sites/default/files/media_asset/20150714_FS_MDG6_Report_en.pdf

2. Manciu C, Largu MA, Vătă, A, Nicolau C., Prisăcariu L, Stoica D, Dorobăț C. Calitatea vieții la pacienții Centrului Regional HIV/SIDA din Iași. *Rev Med Chir.* 2011;115(4):1214-1218.
3. Benea OE, Streinu Cercel A (2011) *Managementul bolnavului cu infecție HIV*, IBI Matei Balș, București.
4. CDC Atlanta, Georgia. A Cluster of Kaposi's Sarcoma and Pneumocystis carinii Pneumonia among Homosexual Male Residents of Los Angeles and range Counties, California. *MMWR* June 18, 1982; 31(23):305-307. Online at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/00001114.htm> (Accessed on Nov 25, 2015)
5. *Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents*. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Online at: http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_o.pdf (Accessed on Nov 25, 2015)
6. Manciu C, Dorobat C, Astarastoe V, Largu MA. The HIV-positive patient in intensive care – psychological profile, *Rev Med Chir*, 2014;118(3):738-743.
7. Manciu C, Dorobat C, Dănilă C, Largu MA. Lymphoma in an HIV-positive patient, *Rev Med Chir*, 2015;119(1):97-100.
8. Talam NC, Gatongi P, Rotich J, et. al. Factors affecting antiretroviral drug adherence among HIV/AIDS adult patients attending HIV/AIDS clinic at Moi Teaching and Referral Hospital, Eldoret, Kenya. *East Afr J Public Health* 2008;5(2):74-78.

NEWS

ASPIRIN USE AND COLORECTAL CANCER

Colorectal cancer (CRC) is the second most frequent type of cancer in Europe, with higher incidence for men after 50 years of age. Aspirin has anticancer effects. The aspirin use reduced the colorectal adenomas risk and the distant metastasis for patients with CRC. A recent meta-analysis summarized the results of eleven studies and showed that aspirin therapy after CRC diagnosis improved survival particular among patients with positive prostaglandin endoperoxide synthase 2 (PTGS2) expression and mutated PIK3CA tumors. PTGS2 is an enzyme involved in prostaglandin synthesis which promote inflammation and cell proliferation. Phosphatidylinositol 3-kinase (PI3K) has an important role in carcinogenesis. Also, the meta-analysis highlighted no association between aspirin use before diagnosis and CRC mortality. Future research is needed in order to establish the optimal doses and duration of aspirin adjuvant therapy in CRC (Peiwei Li; Han Wu; Honghe Zhang; Yu Shi; Jinming Xu; Yao Ye; Dajing Xia; Jun Yang; Jianting Cai; Yihua Wu. Aspirin use after diagnosis but not pre-diagnosis improves established colorectal cancer survival. *Gut* 2015;64(9):1419-1425).

Cătălina Luncă

CONCENTRATIONS OF VITREAL CYTOKINES IN RHEGMATOGENOUS RETINAL DETACHMENT

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CONCENTRATIONS OF VITREAL CYTOKINES IN RHEGMATOGENOUS RETINAL DETACHMENT (Abstract): Proliferative vitreoretinopathy (PVR) is one of the most frequent causes of failure of rhegmatogenous retinal detachment (RRD) surgery. **Aim:** To measure the vitreous levels of granulocyte colony stimulating factor (G-CSF) and monocyte chemoattractant protein 1 (MCP-1) in eyes with RRD and in a control group. **Material and methods:** A prospective study of 40 patients operated for RRD (study group) and 20 patients with epiretinal membrane or macular holes (selected as control group since they needed vitrectomy but had attached retinas). Vitreous samples were collected during vitrectomy and were assessed for the presence of cytokines using a fluorescent bead-based multiplex assay. **Results:** The concentration of G-CSF (8.59 pg/ml) and MCP-1 (1615.2 pg/ml) were significantly increased in the study group, when compared to the control group (0 and 469.13 pg/ml, respectively). MCP-1 was also significantly increased in the subgroup of patients with PVR compared to the patients with uncomplicated RRD. **Conclusions:** The levels of these biomarkers support the idea that proliferative vitreoretinopathy has an inflammatory component. **Keywords:** RHEGMATOGENOUS RETINAL DETACHMENT, PROLIFERATIVE VITREORETINOPATHY, VITREOUS CYTOKINES.

INTRODUCTION

Proliferative vitreoretinopathy (PVR) is a complication of rhegmatogenous retinal detachment (RRD) characterized by fibrocellular, contractile, epiretinal or subretinal membranes that prevent retinal reattachment. It is one of the most frequent causes of RRD surgery failure (1). Retinal break and subsequent retinal detachment are believed to allow macrophages, retinal pigment epithelial cells, glial cells and fibroblasts to migrate into the vitreous, where

they proliferate, form extracellular matrix and assemble into membranes (2).

Several models of the pathogenesis of this disease have been described, some models focusing on the role of inflammatory cells, others on the role of growth factors and cytokines (2). Numerous authors have identified elevated levels of a variety of growth factors, cytokines and chemokines in the vitreous of eyes with PVR (3).

Cytokines serve as signals between cells and are involved in cell proliferation and

migration, inflammation, angiogenesis and fibrosis. Chemokines are mediators that may promote recruitment of leukocytes to the sites of inflammation, enhance immune responses, and also participate in angiogenesis and fibrosis (4).

Multiplex bead-based flow cytometric assays have the major advantage of assessing the presence of numerous different biomarkers in a small quantity of biological fluid. Several authors believe that multiplex immunoassays show similar performance to ELISA in terms of accuracy and reproducibility and have superior sensitivity. (5).

In the present study we have measured the vitreous concentrations of granulocyte colony stimulating factor (G-CSF) and monocyte chemoattractant protein-1 (MCP-1) in eyes with RRD and in a control group, using a multiplex fluorescent bead-based flow cytometric assay.

MATERIAL AND METHODS

The design of this study was prospective, controlled and observational. The study group consisted of 40 eyes of 40 patients with RRD who underwent 23-G pars plana vitrectomy in the Ophthalmology Department of the Iasi University Hospital, Romania, between March and September 2015. All patients were aged over 18 years and had a primary RRD. There were 30 patients with uncomplicated RD and 10 patients with RD and PVR (4 stage B, 5 stage C and 1 stage D, according to the 1983 Retina Society classification) (6). The exclusion criteria were: history of intraocular surgery in the past 6 months, history of intraocular inflammation, diabetic retinopathy or retinal vascular occlusions, general or intraocular steroids or anti-vascular endothelial growth factor in the past 3 months, impossibility to

visualize the causative retinal break preoperatively.

The control group consisted of 20 patients with macular hole or epiretinal membrane who also underwent pars plana vitrectomy. We have selected these conditions because the retinas are attached but they also require a surgical approach, thus providing an opportunity for the sampling of vitreous.

All patients and controls underwent an ophthalmological examination by the recruiting surgeon, including measurement of visual acuity and intraocular pressure, biomicroscopy of the anterior segment of the eye, biomicroscopy or indirect ophthalmoscopy of the posterior segment.

The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Iasi University of Medicine and Pharmacy. Informed consent was obtained from each patient prior to study initiation.

Surgeries were performed by a single surgeon (C.D.), using local anesthesia, 3-port transconjunctival vitrectomy system (Alcon Constellation, Alcon Laboratories, Alcon, Fort Worth, Texas, USA) and a wide-angle viewing system (EIBOS, Moeller-Wedel GmbH & Co. KG, Wedel, Germany).

The vitreous samples were obtained undiluted by manual suction in a sterile syringe connected to the vitreous cutter, before opening the infusion line. If suction became impossible because of blocking with vitreous gel, the cutter function was activated and the suction continued. The content of the syringe was immediately transferred into labelled 1.5 ml Eppendorf Protein LoBind tubes and frozen at -20° C. The tubes were then stored at -150° C. In all cases, the infusion line was opened

immediately after sample collection and vitrectomy was carried on uneventfully.

All vitreous samples were thawed and centrifuged in September 2015. The concentrations of biomarkers (cytokines, chemokines and growth factors) were measured using a multiplex microbead-based immunoassay designed for cytometry. (Cytometric Bead Array®, BD Biosciences, New Jersey, USA). The capture beads corresponding to each analyte to be measured were mixed together in wash buffer, centrifuged, the supernatant was discarded and the beads were resuspended in diluent to a final concentration of 50 µl/test. 50 µl of the supernatant from the centrifuged samples were incubated in labelled tubes with 50 µl mixed capture beads for 1 hour at room temperature. 50 µl of phycoerythrin (PE) detection reagent were added in each tube and then incubated for 2 hours. After washing away the unbound PE and resuspension in wash buffer, the samples were acquired on a FACSAria™ flow cytometer platform. Each bead population has distinct fluorescence intensity, permitting simultaneous measure-

ments of multiple protein concentrations. The median fluorescence intensity of each biomarker was correlated with the dilution of standard biomarkers, the resulting correlation enabling us to quantify the concentration of biomarkers in the study samples. The sensitivity range of each assay was 0-2500 pg/ml.

The statistical analysis was performed using MedCalc (MedCalc Software bvba, Ostend, Belgium). The significance of the difference in concentration of biomarkers between groups was assessed by the Wilcoxon test. A p value of less than 0.05 was considered significant.

RESULTS

The mean granulocyte colony stimulating factor (G-CSF) concentration in the study group was 8.59 ± 28.9 pg/ml (between 0-161.9 pg/ml), whereas all thye eyes in the control group had undetectable levels of G-CSF (0 pg/ml). Since the found values did not have normal distribution, a Wilcoxon test was used to study the difference between groups. The difference was statistically significant, $p=0.02$.

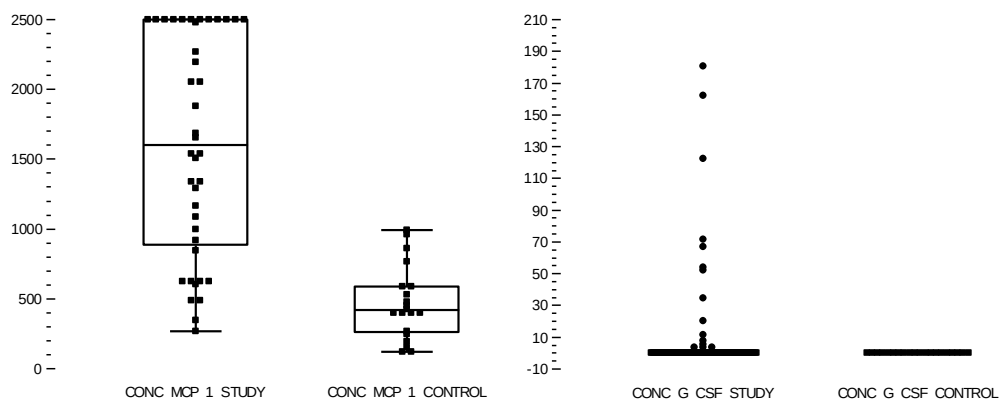


Fig 1. Concentrations of cytokines in the study and control group (pg/ml)

The mean monocyte chemoattractant protein-1 (MCP-1) concentrations in the study and control group were 1615.2 ± 790.8 pg/ml (range 270.1- 2500) and 469.13 ± 263.08 pg/ml (range 118.48-990.49), respectively. The Wilcoxon test showed a highly signifi-

cant difference, $p=0.0001$ (fig. 1).

When comparing the concentrations of MCP-1 and G-CSF between the PVR and non-PVR group we found a significant increase of MCP-1 in the PVR group (but not of G-CSF). (tab. I)

TABLE I

Concentration of G-CSG and MCP-1 in retinal detachment – comparison between eyes with or without proliferative vitreoretinopathy.

	Mean concentration, RRD without PVR (pg/ml)	Mean concentration, RRD with PVR (pg/ml)	Wilcoxon test p value
G-CSF	4.09 ± 14.31 (0-71)	22.12 ± 51.92 (0-161.9)	0.6
MCP-1	1451.22 ± 795.15 (270.16-2500)	2107.46 ± 560.4 (597.3-2500)	0.029

DISCUSSION

The course of PVR is the result of the balance (or disruption of balance) between the destructive and protective mechanisms triggered by the occurrence of a retinal break.

The clinical features of PVR corresponds to the histopathological findings, represented by fibrocellular (and, in evolution, contractile) membranes on the anterior or posterior retinal surface (3).

The primordial element seems to be partial de-differentiation, migration and proliferation of retinal pigment epithelial (RPE) cells, creating areas of hyperplasia, first at the limit between detached and attached retina and at the margins of retinal breaks. This process may start 3 days after retinal detachment begins. The next step is the activation of glial cells, with proliferation of astrocytes, Muller cells, microglia and capillary endothelial cells (1).

The role of growth factors, cytokines and chemokines is highlighted by an ever-growing body of literature, the sampling of vitreous and subretinal fluid being relatively easy during modern vitreoretinal surgery. The measuring of different factors is

also made easier by tests that use minute quantities of biological material.

In our study group of eyes with rhegmatogenous retinal detachments we found a significant increase in the concentrations of G-CSF and MCP-1 (when compared with a control group of eyes with macular holes or idiopathic epiretinal membranes).

Our findings support the data published by Kunikata et al. (8) who have also found that MCP-1/CCL2 and G-CSF were significantly elevated in eyes with RRD, while TNF was not. They have reported that intravitreal triamcinolone is able to suppress elevated levels of intraocular MCP-1 in eyes with RRD. MCP-1 (also known as C-C motif ligand 2) is a chemokine that recruits monocytes, memory T cells and dendritic cells to sites of tissue injury (4). In animal models of retinal detachment, it is expressed in the Muller glia and macrophage/microglia. It may contribute to monocyte recruitment in areas of RRD, and appears to be implicated in the pathogenesis of photoreceptor degeneration and apoptosis following retinal detachment (9).

Yoshimura et al. (4) have also reported that MCP-1 was significantly elevated in

eyes with diabetic macular edema, proliferative diabetic retinopathy, vein occlusion or rhegmatogenous retinal detachment. They have found that MCP-1, IL6 as well as IL8 are increased.

G-CSF is a cytokine that stimulates the survival, proliferation and function of neutrophil precursors and mature neutrophils. In an experimental setting, vitreous from eyes with proliferative vitreoretinopathy stimulated G-CSF and IL-17A production by ARPE-19 cells (10). G-CSF has been shown to have a neuroprotective effect after retinal ganglion cell axotomy (11)

In a retrospective case-control study, Ricker et al. (12) have compared the concentration of 50 biomarkers in the vitreous of patients with uncomplicated RRD and patients who developed PVR within the first 3 months after surgery. They have found a significant increase of IL2, MCP-1/CCL2 and other 15 biomarkers in the vitreous of patients that developed PVR. They found no differences for the values of IL4, IL10, IL12p70, VEGF, bFGF, TNF and other 17 biomarkers (however, they did not provide values for the biomarkers that did not differ between the study and control

group). Banerjee et al. (13) promoted the idea that the detectable levels of interleukins and chemokines support the role of an inflammatory response in PVR.

CONCLUSIONS

In our study group of patients with rhegmatogenous retinal detachment, the concentrations of G-CSF and MCP-1/CCL2 are significantly increased when compared with a control group. A significant increase of MCP-1 in eyes with proliferative vitreoretinopathy compared to eyes with uncomplicated retinal detachments was also found, supporting the hypothesis of the implication of this soluble factor in the pathogeny of PVR.

Acknowledgements

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REFERENCES

1. Rouberol F, Chiquet C. Proliferative vitreoretinopathy: pathophysiology and clinical diagnosis. *J Fr Ophthalmol*, 2014;37(7):557-565
2. Moysidis SN, Thanos A, Vavvas DG. Mechanisms of inflammation in proliferative vitreoretinopathy: from bench to bedside. *Mediators Inflamm*, 2012;2012: 815937.
3. Garweg JG, Tappeiner C, Halberstadt M. Pathophysiology of proliferative vitreoretinopathy in retinal detachment. *Surv Ophthalmol*, 2013; 58(4):321-329.
4. Yoshimura T, Sonoda KH, Sugahara M et al. Comprehensive analysis of inflammatory immune mediators in vitreoretinal diseases. *PLoS One*, 2009 Dec 4;4(12)
5. de Jager W, Bourcier K, Rijkers GT, et al. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol*, 2009 28; 10:52.
6. Retina Society Terminology Committee. The classification of retinal detachment with proliferative retinopathy. *Ophthalmology*, 1983;90(2):121-125

7. Dai Y, Wu Z, Sheng H et al. Identification of inflammatory mediators in patients with rhegmatogenous retinal detachment associated with choroidal detachment. *Mol Vis*, 2015 Apr 10;21:417-427.
8. Kunikata H, Yasuda M, Aizawa N et al. Intraocular concentrations of cytokines and chemokines in rhegmatogenous retinal detachment and the effect of intravitreal triamcinolone acetate. *Am J Ophthalmol*, 2013; 155(6):1028-1037.
9. Nakazawa T, Hisatomi T, Nakazawa C et al. Monocyte chemoattractant protein 1 mediates retinal detachment-induced photoreceptor apoptosis. *Proc Natl Acad Sci U S A*, 2007 13;104(7):2425-2430
10. Bastiaans J, van Meurs JC, Mulder VC et al. The role of thrombin in proliferative vitreoretinopathy. *Invest Ophthalmol Vis Sci*, 2014 11;55(7):4659-4666.
11. Frank T, Schlachetzki JC, Görnicke B et al. Both systemic and local application of granulocyte-colony stimulating factor (G-CSF) is neuroprotective after retinal ganglion cell axotomy. *BMC Neurosci*, 2009 14; 10:49-53.
12. Ricker LJ, Kessels AG, de Jager W, et al. Prediction of proliferative vitreoretinopathy after retinal detachment surgery: potential of biomarker profiling. *Am J Ophthalmol*, 2012 ; 154(2):347-354.
13. Banerjee S, Savant V, Scott RA et al. Multiplex bead analysis of vitreous humor of patients with vitreoretinal disorders. *Invest Ophthalmol Vis Sci*, 2007; 48(5):2203-2207.

NEWS

DREAM OR REALITY? VIDEO HEAD IMPULSE TEST READY TO BE USED

Looking in the past, over the history of vestibulology, the aim was always to measure the function of all the vestibular receptors. Vestibular evoked myogenic potentials (cervical and ocular) show the activity of the saccule and the utricle. Now, using video Head Impulse Test is possible quantify the semicircular canals function by measuring the eye rotation response to an abrupt head rotation in the plane of the canal. The main measure of canal adequacy is the ratio of the eye movement response to the head movement stimulus i.e. the gain of the vestibulo-ocular reflex (VOR). However there is a need for normative data about how VOR gain is affected by age and also by head velocity, to allow the response of any particular patient to be compared to response of healthy subjects in their age range. This study held on healthy subjects divided in decade age groups. VOR gain decreased at high head velocities, but was largely unaffected by age (McGarvie LA, MacDougall HG, Halmagyi GM, Burgess AM, Weber KP, Curthoys IS. The Video Head Impulse Test (vHIT) of Semicircular Canal Function – Age-Dependent Normative Values of VOR Gain in Healthy Subjects. *Frontiers in Neurology* 07/2015; 6:154.)

Maria Cristina Hera

TREATMENT OF PUBIC DISJUNCTION IN YOUNG-BURGUESS TYPE II AND III PELVIC RING FRACTURES

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TREATMENT OF PUBIC DISJUNCTION IN YOUNG-BURGUESS TYPE II AND III PELVIC RING FRACTURES (Abstract). **Aim:** To present the personal experience in assessing the treatment of pubic disjunction in Young-Burguess type II and III pelvic ring fractures. **Material and methods:** Included in the study were 30 patients with type II and III pelvic ring fractures, aged 32 to 76 years. Of these, 18 patients were treated with external fixation, and 12 patients were treated surgically: type II fractures were treated with open reduction and internal fixation (ORIF) of pubic disjunction and type III fractures underwent additionally posterior fixation. **Results:** Mean follow-up was 1.6 years. After external fixation we found the persistence of diastasis (1.5 to 4 cm), which was asymptomatic in all cases. Complications included implant failure in 3 patients, and postoperative infection in 2 patients. Among the 5 patients with type II lesions the clinical and radiological scores were excellent in 3, good in 1, and poor in 1. Among the patients with type III lesions the clinical outcomes were excellent in 5 and good in 2. **Conclusions:** External fixation has proved a good treatment option for patients who cannot tolerate a more extensive surgery. There is no conclusive clinical evidence favoring the use of two perpendicular plates vs. 1 plate for pubic symphysis fixation. In osteoporotic patients the use of 2 plates is recommended. **Key-words:** PELVIC RING FRACTURE, PUBIC DISJUNCTION, ORIF.

Pubic disjunction result from high-energy trauma, and occurs in 14 - 20% of pelvic ring fractures (1). They are frequently associated with multiple other lesions, such as blood vessels rupture with massive hemorrhage, this association having a mortality rate of approximately 15-35% (1, 2). The major sources of bleeding are supposed to be the presacral and retroperitoneal blood vessels. (1).

The conservative treatment of these fractures has proved ineffective. An early firm stabilization and anatomic reduction

are recommended to decrease mortality and allow early mobilization (3).

To stabilize these fractures several methods are available. External fixators can be used for the temporary stabilization of all types of pelvic ring fractures or for a definitive one in some cases. Anterior internal fixation can be used as a definitive treatment for pubic disjunctions if the sacroiliac ligaments are intact. Anterior internal fixation may be associated with posterior fixation in patients with posterior pelvic ring injuries (especially in APC type

III) to improve overall stability (4).

MATERIAL AND METHODS

In the interval 2008-2014 in the orthopedics and traumatology units of the Iasi "Sf. Spiridon" Emergency County Hospital and Botosani "Mavromati" Emergency County Hospital 30 patients with type II and III pelvic ring fractures, aged between 32 and 81 years (mean age 55 years) were treated. The mechanisms of fracture were: motor vehicle accidents (17: 56,66%), falls from height (10: 33,33%) and 3 (10%) by crush injury (3). The injuries most commonly associated with these fractures were: head trauma, craniofacial trauma, thoracoabdominal trauma, splenic

rupture, bladder rupture, lower leg fracture, forearm fracture, fractured clavicle and humerus.

Eighteen patients were treated with external fixation (fig. 1) and 12 patients were treated surgically: type II fractures were treated with ORIF of pubic disjunction and type III fractures underwent additionally posterior fixation. Pubic disjunctions in type II lesions were fixed with one 3.5 mm reconstruction plate and 4 screws (2 cases/16.6%) and with two perpendicular plates (3 cases/25%); those in type III lesions were treated with one 3.5 mm reconstruction plate and 4 screws (3 cases/25%) and two 3.5 mm reconstruction plates (4 cases/33.33%) (fig. 2).

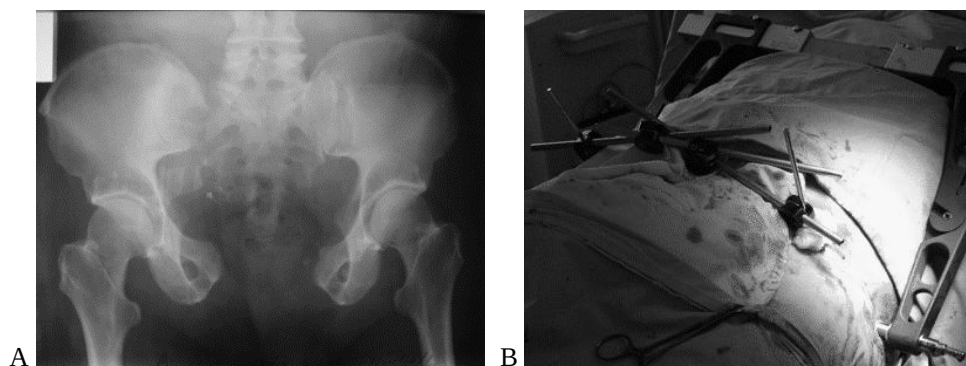
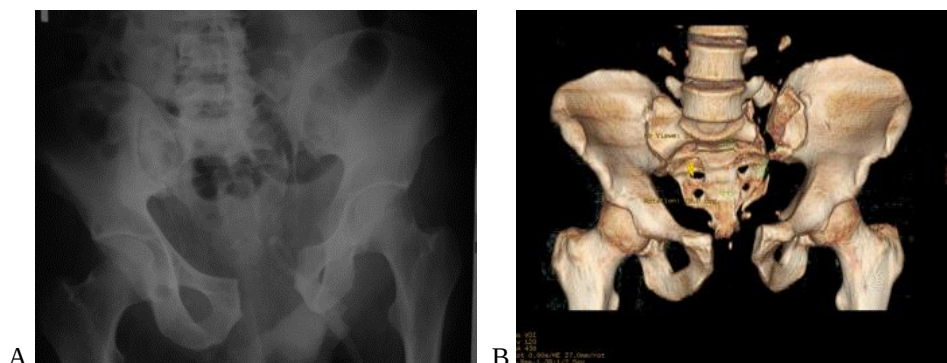


Fig. 1. – patient B.I., 49 years old, victim of motor vehicle accident. A – preoperative X ray – APC type III injury, B – intraoperative view – posterior stabilization with „C clamp” and anterior with supraacetabular external fixation.



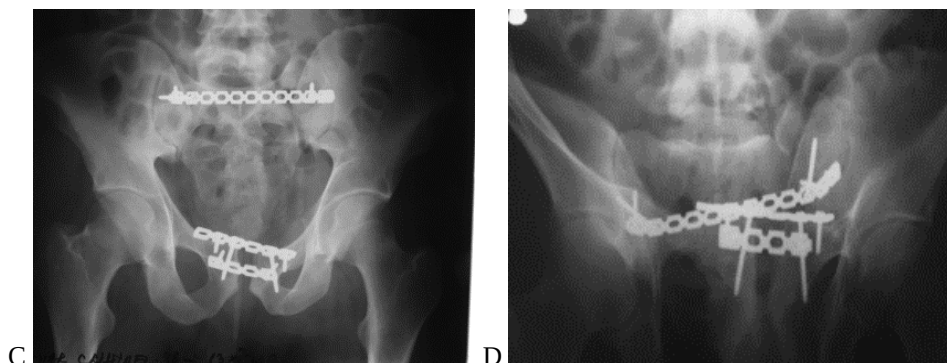


Fig. 2. – patient L.M., 55 years old, victim of motor vehicle accident. A – preoperative X ray – APC type III injury, B – 3D image - APC type III injury, C–postoperative anterior X ray – fixation with 1 plate posteriorly and 2 plates anteriorly, D– postoperative "outlet" X ray – fixation with 1 plate posteriorly and 2 plates anteriorly

RESULTS

Mean follow-up was 1.6 years. The radiologic score and Majeed clinical score were used to assess the outcomes. After external fixation we found the persistence of diastasis (1.5 to 4 cm), which was asymptomatic in all cases (tab.I, II).

TABLE I

Interpretation of the radiologic score

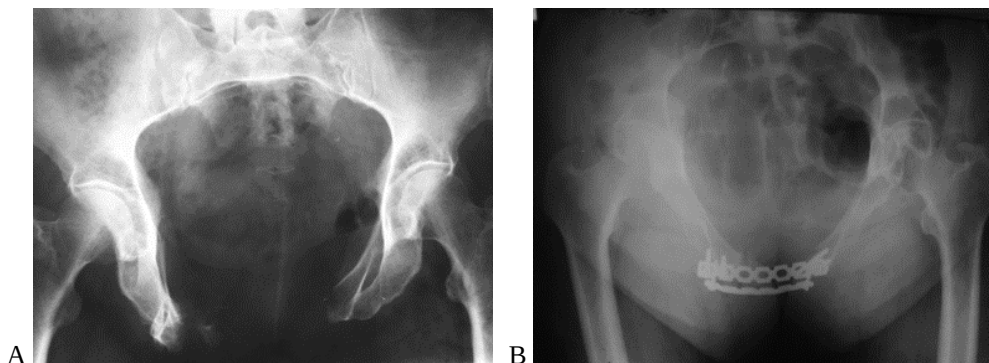
RESULT	RESIDUAL DISPLACEMENT
Excellent	0-5 mm
Good	6-10 mm
Fair	11-15 mm
Poor	>15 mm

TABLE II

Interpretation of the clinical score

RESULT	TOTAL SCORE
Excellent	78-80
Good	70-77
Fair	60-69
Poor	<60

Among the 12 patients treated surgically (ORIF) the complications included construct failure (3:25%) (fig. 3) and postoperative infection (2:16.6%). In the 5 (41,66%) patients with type II lesions, clinical scores were excellent in 3 cases (60%), good in 1 (20%) and poor in 1 (20%).



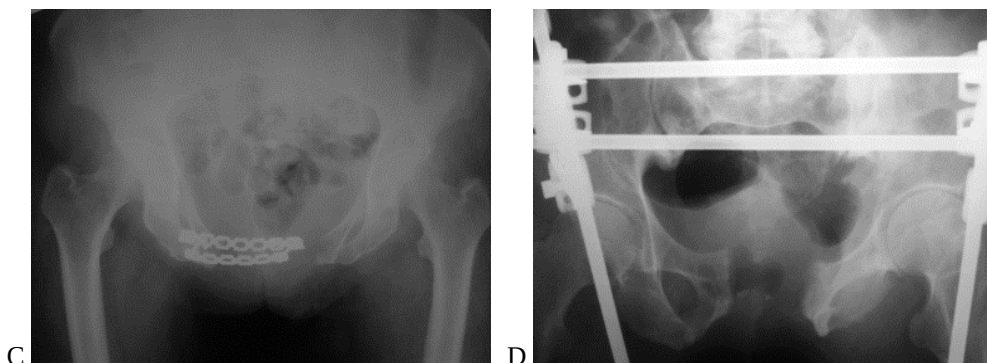


Fig. 3. – patient M.E., 76 years old, victim of motor vehicle accident. A – preoperative X ray – APC type II injury, B – postoperative "inlet" view – fixation with 2 plates anteriorly, C– X ray on postoperative day 7 – loss of reduction, D– postoperative X ray – stabilization with external fixator.

Radiological scores were excellent in 3 (60%) cases, good in 1 (20%), and poor in 1 (20%). Of the patients with type III injuries

5 (71.43%) had excellent clinical outcomes and 2 (28.57%) good. X-ray results were similar to those in type II injuries (tab. III).

TABLE III

Profile of the patients treated surgically by open reduction and internal fixation.

No.	Age	Injury type	Mechanism	Fixation	Radiologic score	Clinical score
1	32	APC II	Vehicle accident [passenger]	Anterior [1 plate]	8 mm	70
2	38	APC III [sacral fracture]	Vehicle accident [motorcyclist]	Anterior [1 plate] + Posterior [1 plate]	5 mm	78
3	61	APC III [sacral fracture]	Vehicle accident [passenger]	Anterior [1 plate]+ Posterior [2 screws]	10 mm	70
4	40	APC III [ligament injury]	Fall from height	Anterior [2 plates] + Posterior [2 screws]	4 mm	77
5	48	APC II	Vehicle accident [passenger]	Anterior [1 plate]	5 mm	80
6	55	APC III [sacral fracture]	Vehicle accident [passenger]	Anterior [2 plates] + Posterior [1 plate]	4 mm	78
7	34	APC II	Vehicle accident [passenger]	Anterior [2 plates]	4 mm	77
8	38	APC II	Vehicle accident [motorcyclist]	Anterior [2 plates]	5 mm	78
9	67	APC III [sacral fracture]	Vehicle accident [pedestrian]	Anterior [1 plate] + Posterior [1 plate]	17 mm	74
10	35	APC III [ligament injury]	Vehicle accident [motorcyclist]	Anterior [2 plates] + Posterior [2 screws]	5 mm	79
11	76	APC II	Vehicle accident [pedestrian]	Anterior [2 plates]	36 mm	56
12	36	APC III [ligament injury]	Fall from height	Anterior [2 plates] + Posterior [1 screw]	5 mm	78

DISCUSSIONS

With the progress made in the emergency care system, prompt diagnosis, resuscitation methods, better management of visceral lesions and improved imaging techniques, the orthopedic surgeon plays a key role in the treatment of these patients (3).

In cases of stable fractures without disjunction and stable ligamentous structures a conservative treatment is accepted with good outcomes. Unstable lesions usually require surgical treatment of the anterior and posterior ring, if the latter is injured (4).

In our study external fixation proved to be a good treatment option for patients who cannot tolerate a more extensive surgery.

Management of APC type II pelvic ring fractures with external fixation is effective, relatively simple, minimally invasive, with less intraoperative blood loss and shorter surgical time and allows earlier patient mobilization (5).

A retrospective study of 64 supraacetabular external fixators to stabilize anterior pelvic ring showed iatrogenic lesion of the lateral femoral cutaneous nerve (4.5%) which reversed within 1 year, no pin site infection, no secondary displacements, perforation of the pin into the small pelvis requiring no treatment in 3% of cases, and pseudoarthrosis of the pubic and ischial rami requiring surgical treatment in 1 case (6). The temporary use of an external fixator is safe and effective, but its use for a definitive treatment is associated with high infection rate and secondary displacements according to the study conducted by Mason (7). Galois L (8) says that anterior inferior placement of the external fixator showed a potential for increased stability.

Pubic disjunctions > 2.5 cm reflect rotational instability and benefit from anterior

stabilization, internal fixation being preferred to external fixation. There is no universally accepted protocol on the type of plate (dynamic compression plate vs. pelvic reconstruction plate), plate thickness (3.4 vs. 4.5 mm) or number of plate screws. There is conflicting evidence on the benefits or need to use a 2-hole plate. It has been agreed that additional posterior instability requires a surgical posterior stabilization procedure (4).

In our surgically treated patients we thought that in case of APC type III injuries anterior and posterior stabilization is mandatory. As to the stabilization of pubic disjunction, no differences between the number of used plates (1 plate vs 2 plates) and clinical and radiological scores were found. However, it is worth mentioning that in osteoporotic patients using a single plate does not provide a satisfactory stabilization.

Initially based on biomechanical studies it was considered that adding a second plate can lead to better stabilization, theoretically being indicated in unstable fractures (9).

In a retrospective study Bagchi K. (4) found no conclusive evidence favoring the use of two plates *versus* one plate to stabilize the pubic symphysis. Several clinical and biomechanical studies describe the use of a single plate as appropriate for all types of pelvic ring fractures.

Although plate fixation of pubic disjunctions is the recommended treatment, the incidence and consequences of fixation failure remained a concern. Some of these are due to osteosynthesis material failure and low bone density. However, these do not seem to affect the clinical outcome, because few patients require surgical re-intervention (2).

A study evaluating locked versus non-locked plate osteosynthesis of the pubic symphysis showed that locked plates together with the required screws have no advantage over non-locked plates, but further studies are needed (10). On the other hand, Pizanis (11) suggested a biomechanical advantage in using anatomically contoured plates with locking screw capabilities in case of large displacement or obese patients. A biomechanical cadaveric study conducted in 2013 revealed that locked plate osteosynthesis at the pubic symphysis has no advantage over non-locked plates (12).

Lange (13) compared osteosynthesis with a two-hole anterior plate versus two plates (one superior and the other anterior) for pubic disjunction. Double plate osteosynthesis offers a more rigid fixation, but the procedure requires extensive dissection which may cause a significant loss of blood. Single plate osteosynthesis is less

rigid and helps accommodate the normal movement of the pubic symphysis without loss of reduction and with a limited dissection.

Simonian (14) and Dujardin (15) in their studies concluded that osteosynthesis of the anterior and posterior pelvic ring is optimal for APC II and III injuries as compared to single plate anterior pelvic ring fixation.

CONCLUSIONS

The results of this study allow us to state that external fixation has proved a good treatment option in patients who cannot tolerate a more extensive surgery. There is no conclusive clinical evidence favoring the use of two perpendicular plates vs. 1 plate for pubic symphysis fixation; in osteoporotic patients the use of 2 plates is indicated regardless of lesion type. A residual diastasis below 1 cm is accompanied by good clinical outcomes.

REFERENCES

1. Sellei RM, Schandelmaier P, Kobbe P, Knobe M, Pape HC. Can a modified anterior external fixator provide posterior compression of AP compression type III pelvic injuries? *Clin Orthop Relat Res.* 2013; 471(9): 2862-2868.
2. Morris SA, Loveridge J, Smart DK, Ward AJ, Chessier TJ. Is fixation failure after plate fixation of the symphysis pubis clinically important? *Clin Orthop Relat Res.* 2012; 470(8): 2154-2160.
3. Choy WS, Kim KJ, Lee SK, Park HJ. Anterior pelvic plating and sacroiliac joint fixation in unstable pelvic ring injuries. *Yonsei Med J.* 2012; 53(2): 422-426.
4. Bagchi K, Uhl RL. Fixation of pubic symphyseal disruptions: one or two plates? *Orthopedics.* 2009; 32(6): 427-432.
5. Alexa O, Iancu C, Veliceasa B, Puha B, Georgescu N. Results of external fixation in pelvic ring fractures. *Rev Med Chir Soc Med Nat Iasi.* 2007;111(2): 428-434.
6. Gänsslen A, Pohlemann T, Krettek C. A simple supraacetabular external fixation for pelvic ring fractures. *Oper Orthop Traumatol.* 2005; 17(3): 296-312.
7. Mason WT, Khan SN, James CL, Chessier TJ, Ward AJ. Complications of temporary and definitive external fixation of pelvic ring injuries. *Injury.* 2005; 36(5): 599-604.
8. Galois L, Pfeffer F, Mainard D, Delagoutte JP. The value of external fixation for unstable pelvic ring injuries. *Acta Orthop Belg.* 2003; 69(4): 321-327.

9. Meissner A, Fell M, Wilk R, Boenick U, Rahmzadeh R. Comparison of internal fixation methods for the symphysis in multi-directional dynamic gait simulation. *Unfallchirurg*. 1998; 101(1): 18-25.
10. Prasarn ML, Zych G et al. Biomechanical study of 4-hole pubic symphyseal plating: locked versus unlocked constructs. *Orthopedics*. 2012 1; 35(7): e1028-1032.
11. Pizanis A, Garcia P, Santelmann M, Culemann U, Pohlemann T. Reduction and fixation capabilities of different plate designs for pubic symphysis disruption: a biomechanical comparison. *Injury*. 2013 ; 44(2): 183-188.
12. Moed BR, O'Boynick CP, Bledsoe JG. Locked versus standard unlocked plating of the symphysis pubis in a Type-C pelvic injury: a cadaver biomechanical study. *Injury*. 2014; 45(4): 748-751.
13. Lange RH, Hansen ST Jr. Pelvic ring disruptions with symphysis pubis diastasis. Indications, technique, and limitations of anterior internal fixation. *Clin Orthop Relat Res*. 1985; (201): 130-137.
14. Simonian PT, Routt ML Jr. Biomechanics of pelvic fixation. *Orthop Clin North Am*. 1997; 28(3): 351-367.
15. Dujardin FH, Roussignol X, Hossenbaccus M, Thomine JM. Experimental study of the sacroiliac joint micromotion in pelvic disruption. *J Orthop Trauma*. 2002; 16(2): 99-103.

NEWS

HEART RATE VARIABILITY IN OVERWEIGHT HEALTH CARE STUDENTS: CORRELATION WITH VISCERAL FAT

Overweight and obesity are due to abnormal or excessive fat accumulation that may impair health and it is because of an imbalance between calories consumed and expended. The global epidemic of overweight and obesity is the major public health problem in developed as well as developing world. Abdominal obesity, defined as increased waist circumference is one of the components of the constellation of metabolic abnormalities collectively called as the metabolic syndrome (MS). The latest definition of MS by the International Diabetes Federation (IDF) has included abdominal obesity as one of the essential components. Among the different available non-invasive techniques for assessing the autonomic status, Heart Rate Variability (HRV) is a simple method to evaluate the sympathovagal balance at the sinoatrial level. Increased sympathetic activity, decreased parasympathetic activity and sympathetic imbalance (SVI) has been reported in obese individuals. However, the SVI and its association with visceral fat in overweight health care students have not been explored. Therefore, in the present study, was observed the sympathovagal imbalance due to increased sympathetic activity and its association with visceral fat in overweight individuals (Chintala KK, Krishna BH, Reddy MN. Heart Rate Variability in Overweight Health Care Students: Correlation with Visceral Fat. *Journal of Clinical and Diagnostic Research*. 2015, Vol-9(1): 6-8).

Doina Butcovan

EFFECT OF TRANEXAMIC ACID USE ON POSTOPERATIVE BLOOD LOSS IN TOTAL KNEE ARTHROPLASTY

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EFFECT OF TRANEXAMIC ACID USE ON POSTOPERATIVE BLOOD LOSS IN TOTAL KNEE ARTHROPLASTY (Abstract). **Aim:** To monitor the effect of tranexamic acid use (Exacyl) on postoperative bleeding (aspiration), autologous blood transfusion (autotransfusion), allogeneic blood transfusion and postoperative anemia (difference between preoperative and immediate postoperative hemoglobin levels) in total knee arthroplasty. **Material and methods:** This retrospective observational study was conducted on a sample of 457 patients who underwent endoprosthetic knee arthroplasty in the interval January 1, 2008-July30, 2014 at the Traumatology-Orthopedics Clinic of the Iasi Rehabilitation Hospital. The mean age of the study group was 66 years (range 32-84 years), 74.4% were female, 39.8% presented *varus* and 6.1% *valgus* malalignment. The study group was subdivided into two groups: **Group 1** served as controls and underwent surgery without the administration of tranexamic acid and **Group 2** received tranexamic acid. **Results:** In group 1 the average amount of blood lost was significantly higher than in group 2, 1168.94 ml vs 452.9 ml ($p < 0.001$). The use of a cell saver was required in 70% of group 1 patients with an average amount of auto transfused blood of 480.70 ml, significantly more frequent and in greater amounts than in group 2 (below 10%) with an average of 15.05 ml ($p < 0.001$). In this study tranexamic acid had not a statistically significant favorable effect on blood transfusion requirements and anemia syndrome. **Conclusion:** Tranexamic acid has a favorable effect on postoperative blood loss and its associated complications, with a favorable impact on both early clinical and functional recovery. **Keywords:** TRANEXAMIC ACID, TOTAL KNEE ARTHROPLASTY, TOURNIQUET, BLOOD LOSS.

Knee osteoarthritis is one of the most common causes of pain and loss of joint function, the risk of disability being as high as that attributable to cardiovascular diseases and higher than in any other disease in the elderly (1). The disease is more common in women (2). In the advanced stages of knee osteoarthritis endoprosthetic arthroplasty is the gold standard treatment, the technique being customized according to the associated deformity and

individual joint architecture (bone and ligaments) (3).

The use of a tourniquet induces local fibrinolysis in the operated lower leg and increased fibrinolytic activity causes significant postoperative blood loss. Blood loss causes such postoperative complications as anemia (4), cardiovascular complications, post transfusion reactions (post transfusion shock, transfusion transmitted diseases, postoperative infection) (5.6), with a direct

impact on the early postoperative course and functional recovery. Tranexamic acid, a synthetic analogue of lysine which competitively inhibits the activation of plasminogen to fibrin (7.8) is used to reduce postoperative blood loss. The aim of this study was to assess the effect of tranexamic acid (Exacyl) use on postoperative bleeding (aspiration), autologous blood transfusion (use of cell saver) and allogeneic blood transfusion on postoperative anemia syndrome (difference between preoperative and immediate postoperative Hb levels, *D* Hb).

MATERIALS AND METHODS

The retrospective observational study was conducted on a sample of 457 patients diagnosed with advanced knee osteoarthritis who underwent endoprosthetic knee arthroplasty in the interval January 1, 2008 - July 30, 2014 at the Traumatology-Orthopedics Clinic of the Iasi Rehabilitation Hospital. The mean age of the study patients was 66

years (range 32-84 years), 74.4% of them being female. The study group was subdivided into two groups: group 1 (78.11%) - patients who were not given Exacyl and group 2 (21.89%) - patients who received intravenous tranexamic acid intraoperatively and immediately postoperatively.

Also evaluated was blood loss depending on the presence or absence of frontal plane deformities, as evidenced by the calculation of hip-knee-ankle (HKA) angle on long leg X- ray (fig.1.a, b). 53.6% of the patients had proper knee alignment, while varus and valgus malalignment was present in 39.8% and 6.1%, respectively.

Sagittal plane malalignment, in flexum, was associated with varus misalignment in 97% of the cases, being the sole finding in only two cases. Of the study patients with genu varum 44% presented a mild deformity of 8 degrees and 18% of over 15 degrees. In genu valgum patients, the three categories were in almost identical percentages.

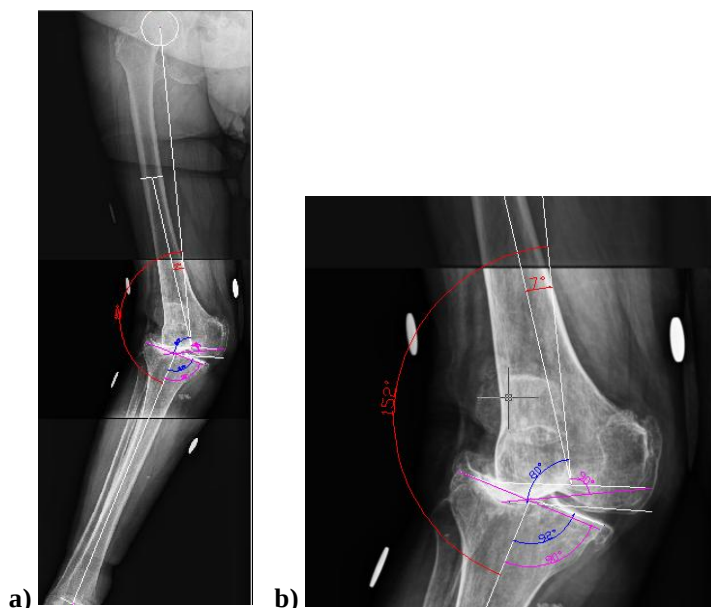


Fig. 1. a) Preoperative planning: Digital long leg X-ray of the lower limb in upright position; b) Advanced secondary knee osteoarthritis on *genu valgum* (HKA- 152°)

RESULTS

1. **Aspiration:** Comparing the blood loss in the two groups we found a statistically significant difference ($p < 0.001$). If in group 1 the average blood loss was of 1168.94 ml (highest: 4150 ml), the analysis of data from group 2 showed an average blood loss of 452.9 ml (highest: 1100 ml) (fig. 2.) Blood loss was not influenced either by age ($p = 0.336$) or sex parameters ($p = 0.512$).

2. **Autotransfusion:** The need for a cell-saver and the amount of autotransfused blood were significantly higher in group 1 ($p < 0.001$). In group 1 a cell-saver was used in 70% of the cases and the average amount of autotransfused blood was 480.70 ml. In group 2 the average amount of autotransfused blood was of only 15.05 ml, and cell saver was used in less than 10% of patients.

3. **Blood Transfusion:** The difference between the two groups was not statistically significant ($p = 0.436$). A lower rate of allogeneic blood transfusion was recorded in group 1 (20% of cases, average amount 68.91ml) compared to group 2 (32% of cases, average amount 99.5ml).

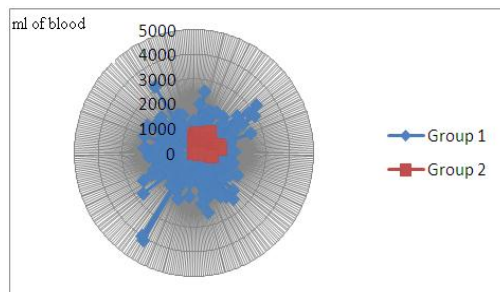


Fig. 2 Graphical representation of blood loss in the two study groups

4. **Anemia syndrome:** Mean *D Hb* was 3.27 mg/dl in group 1 and 2.74 mg/dl in group 2. The highest level was 8.10 mg/dl in group 1 compared to 4.90 mg/dl in group 2. Despite the values differed, the difference did not meet statistical significance ($p = 0.514$).

5. **The presence of deformities:** These variables for quantifying blood loss were also checked within the context of present or absent joint deformities, without noticing significant differences (tab. I). We noted that frontal plane deformities greater than 15 degrees were often associated with an increase in the amount of blood loss.

TABLE I

Average values of blood loss variables according to the presence/absence of diformity

Diformity Y N		No	Mean	Std. Deviation	Std. Error mean
Aspiration	No diformities	245	1001.14	597.456	38.170
	Diformities	212	1025.09	604.920	41.546
Autotransfusion	No diformities	245	357.22	388.491	24.820
	Diformities	212	403.75	387.336	26.602
Transfusion	No diformities	245	77.55	158.522	10.128
	Diformities	212	73.35	149.278	10.252
Hb difference	No diformities	245	3.0718	1.31196	0.08382
	Diformities	212	3.2521	1.24505	0.08551

DISCUSSIONS

Endoprosthetic knee arthroplasty is accompanied by minimal intraoperative bleeding due to the use of pneumatic tour-

net but by important postoperative blood loss.

This study shows that the use of tranexamic acid results in a significant decrease

in postoperative blood loss and hence in the use of cell saver, but has no impact on allogeneic blood transfusion. Our results are in agreement with literature data in terms of the amount of blood collected during suction drainage (4,7,9,10-12) but not in terms of the amount of blood needed for allogeneic blood transfusion (11,12). In our study group the amount of transfused blood did not differ significantly between the two groups, the decision to resort to this therapy being more frequent in group 2, probably due to the fact that cell-saver is more rarely used in this category of patients. It is worth mentioning that although in group 2 the average blood loss was small, anemia syndrome was not statistically more significant in group 1. This mismatch between the amount of drained blood and postoperative anemia raises the suspicion of an additional blood loss. Sehat (13) speaks of a hidden blood loss of 700 ml, probably from hematoma formation.

Tranexamic acid, a synthetic inhibitor of fibrinolysis which acts by competitively blocking the lysine binding sites on plas-

minogen (7), reduces blood loss and financial cost in orthopedic surgery (hip and knee replacement surgery, spinal surgery) (4,9) and cardiovascular surgery (cardiopulmonary bypass) (14) but only insignificantly the hidden blood loss. This phenomenon probably represents the extravasations of red cells after tourniquet release, hemostasis being by primary hemostatic mechanisms, before fibrinolysis has any effects (15).

CONCLUSIONS

Our study highlights the importance of using tranexamic acid in knee arthroplasty as it significantly decreases the postoperative blood loss and financial cost given the fact that the use of cell-saver is less frequently required. This research demonstrates that tranexamic acid does not have a significant impact on allogeneic blood transfusion and anemia syndrome. Tranexamic acid is an essential drug product in total knee arthroplasty as it reduces postoperative blood loss and its associated complications, with a favorable impact on early recovery of joint function.

REFERENCES

1. Guccione AA, Felson DT, Anderson JJ, Anthony JM, Zhang Y, Wilson PW, et al. The effects of specific medical conditions on the functional limitations of elders in the Framingham Study. *Am J Pub Health.* 1994; 84(3):351–358.
2. Carmen Păstrăguș, Codrina Ancuța, Smaranda Ștefana Miu, Iulia Codruța Belibou, Rodica Chiriac: Descriptive clinical study on a sample of patients with gonarthrosis. *Rev. Med. Chir. Soc. Med. Nat.* 2012; 116 (1):34-40.
3. M. Gramada, P. Botez. Influence of preoperative knee deformation on tension force in posterior stabilized total knee arthroplasty. *Rev. Med. Chir. Soc. Med. Nat.* 2012; 116 (3):840-845.
4. Wong J, Abrishami A, El Beheiry H, Mahomed NN, Roderick Davey J, Gandhi R, Syed KA, Muhammad Ovais Hasan S, De Silva Y, Chung F: Topical application of tranexamic acid reduces postoperative blood loss in total knee Arthroplasty: a randomized, double-blinded, placebo-controlled trial of efficacy. *J Bone Joint Surg Am* 2010; 92:2503–2513.
5. Ortega-Andreu M, Pérez-Chrzanowska H, Figueredo R, Gómez-Barrena E: Blood loss control with two doses of tranexamic acid in a multimodal protocol for total knee Arthroplasty. *Open Orthop J* 2011; 5:44–48.

6. Garneti N, Field J: Bone bleeding during total hip arthroplasty after administration of tranexamic acid. *J Arthroplasty* 2004; 19:488–492.
7. Jansen AJ, Andreica S, Claeys M, D’Haese J, Camu F, Jochmans K: Use of Tranexamic acid for an effective blood conservation strategy after total knee arthroplasty. *Br J Anaesth* 1999; 83:596–601.
8. Veien M, Sørensen JV, Madsen F, Juelsgaard P: Tranexamic acid given intraoperatively reduces blood loss after total knee replacement: a randomized controlled study. *Acta Anaesthesiol Scand* 2002; 46:1206–1211.
9. Ralley FE, Berta D, Binns V, Howard J, Naudie DDR: One intraoperative dose of tranexamic acid for patients having primary hip or knee arthroplasty. *Clin Orthop Relat Res* 2010; 468:1905–1911.
10. Hiippala S, Strid L, Wennerstrand M, et al. Tranexamic acid (Cyklocapron) reduces perioperative blood loss associated with total knee arthroplasty. *Br J Anaesth* 1995; 74: 534–537.
11. G, Fredin H. Fibrinolytic inhibition with tranexamic acid reduces blood loss and blood transfusion after knee arthroplasty. *J Bone Joint Surg Br* 1996; 78: 434–440.
12. Hiippala ST, Strid LJ, Wennerstrand MI, et al. Tranexamic acid radically reduces blood loss and transfusions associated with total knee arthroplasty. *Anesth Analg* 1997; 84: 839–844.
13. Sehat KR, Evans R, Newman JH. How much blood is really lost in total knee arthroplasty? Correct blood management should take hidden loss into account. *Knee* 2000; 7: 151–155.
14. Mongan PD, Brown RS, Thwaites BK. Tranexamic acid and aprotinin reduce postoperative bleeding and transfusions during primary coronary revascularization. *Anesth Analg* 1998; 87:258–265.
15. L. Good, E. Peterson, B. Lisander: Tranexamic acid decreases external blood loss but not hidden blood loss in total knee replacement. *Br J Anaesth* 2003; 90: 596–599.

NEWS

ANTIMICROBIAL ROLE OF FILAGGRIN-2 PROTEIN

Filaggrin-2 (FLG2) is a water-insoluble 248 kDa S100 fused-type protein found in the upper epidermis and eccrine sweat glands. A study by Hansmann *et al.* tested the antimicrobial activity of filaggrin-2 against *Pseudomonas aeruginosa* and other Pseudomonads and found that recombinant FLG2 C-terminal protein fragments have potent antimicrobial activity against *P. aeruginosa* and after cultivation of *P. aeruginosa* on stratum corneum, antimicrobially active FLG2 fragments are released from insoluble material by the bacteria themselves. These FLG2 C-terminal fragments have an alternative mode of action different from most other antimicrobial peptides, as they do not induce pore formation, but membrane blebbing. According to Hansmann *et al.*, FLG2 fragments interfere with bacterial replication, inhibit their growth on skin surfaces and contribute to the skin's antimicrobial defense, explaining why *Pseudomonas* infections of healthy skin are very rare. Also, the absence of filaggrin 2 at certain body surfaces and in the case of burned skin seems to be responsible for the higher susceptibility to these infections. The study concluded that FLG2 C-terminal fragments could represent new *Pseudomonas*-targeting antimicrobials (Hansmann B, Schröder JM, Gerstel U. Skin-Derived C-Terminal Filaggrin-2 Fragments Are *Pseudomonas aeruginosa*-Directed Antimicrobials Targeting Bacterial Replication. *PLoS Pathog.* 2015;11(9):e1005159. doi: 10.1371/journal.ppat.1005159).

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OUTCOME OF INTERNAL HEMIPELVECTOMY IN A PATIENT WITH MULTIPLE MYELOMA - CASE REPORT

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OUTCOME OF INTERNAL HEMIPELVECTOMY IN A PATIENT WITH MULTIPLE MYELOMA: CASE REPORT (Abstract). A patient treated for thyroid cancer was diagnosed with multiple myeloma in the context of trauma. After the pelvic tumor devascularization she underwent internal hemipelvectomy Type I + IIA Enneking Classification using polymerizing bionutral gel embolization, "Glubran 2". The purpose of this case report is to demonstrate the importance of careful selection of treatment methods, together with adequate physical therapy in order to obtain favorable long-term results. **Keywords:** HEMIPELVECTOMY, MYELOMA, RECOVERY.

Internal hemipelvectomy is a surgical limb-sparing procedure used to treat malignant tumors of the pelvic ring. The most commonly used classification for hemipelvectomy is that by Enneking classification (1, 2), which distinguishes 4 types depending on the anatomical components removed, combinations of different types being also possible. We report the case of a female patient with multiple myeloma in association with multiple malignancies (3), who underwent Enneking Type I+IIA internal hemipelvectomy on January 21, 2008. The importance of adjuvant treatments and rehabilitation methods on patient outcome is discussed.

CASE REPORT

Patient E.G., 47-year-old female, was diagnosed on October 25, 2004 with nodular goiter and underwent surgical removal of the thyroid tumor. On April 13, 2007,

the patient suffered an injury by falling from a height of about 1m with impact on the right hemipelvis. At physical and radiological examination a massive tumor involving the entire right iliac fossa was detected. The tumor was highly vascularized and its extra-compartmental component was displacing the iliac muscle together with the adjacent arteriovenous vascular groups.

On June 15, 2007 an angiography was performed. It detected a tumor occupying the right iliac fossa, highly vascularized, receiving blood supply from emerging vessels from right hypogastric artery and right lumbar artery IV. Tumor embolization was performed using Glubran 2, a bionutral polymerizing gel, a total tumor devascularization being obtained (figs. 1, 2). On January 21, 2008, after a surgical biopsy was performed, the patient was diagnosed with myeloma. On the same day, the patient un-

derwent internal hemipelvectomy type I (iliac radical resection with gluteal muscles and sciatic nerve preservation) and IIA (excision of the whole acetabulum), according to Enneking classification (fig. 3).

The resection of the right hemipelvis was performed with the following margins 17x16x10 cm, together with the collection of a tumor mass measuring 11x10x9cm, which was consisting of translucent yellow-brown bulky tissue.

Macroscopically, the tumor occupied a part of the iliac fossa, expanding beyond the cortical component to the surrounding soft tissues and reaching the sacroiliac resection edge.

Microscopically, the osteomedullary tissue was infiltrated with a population of plasma cells. Rapid tests, together with immunohistochemistry studies confirmed the diagnosis of myeloma.

After surgery a rehabilitation program

was initiated. After the removal of suture threads, 20 days after surgery, the patient was started on active and passive range-of-motion hip mobilization pool exercises. With the aid of two crutches, the patient resumed walking with left leg weight bearing and right leg in flexion up to 60 degrees, considering the possible risks of early right hip mobilization. A strengthening exercise program for gluteal and right thigh muscles was also started. Forty days after surgery, the patient began the progressive weight bearing on the affected limb. One year after surgery the patient started to walk without using any equipment. The patient presents a leg shortening of 8.7 cm together with a limitation of rotation movements. Up to now, the patient did not present any complication, did not require administration of analgesics, and has already exceeded the average survival rate, seven years after surgery.

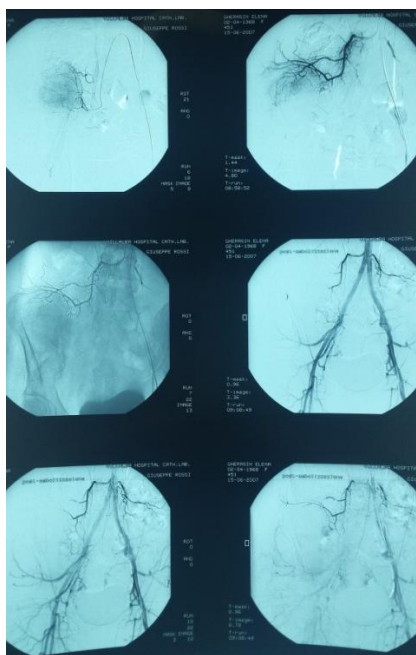


Fig 1- Angiography showing tumor blood supply

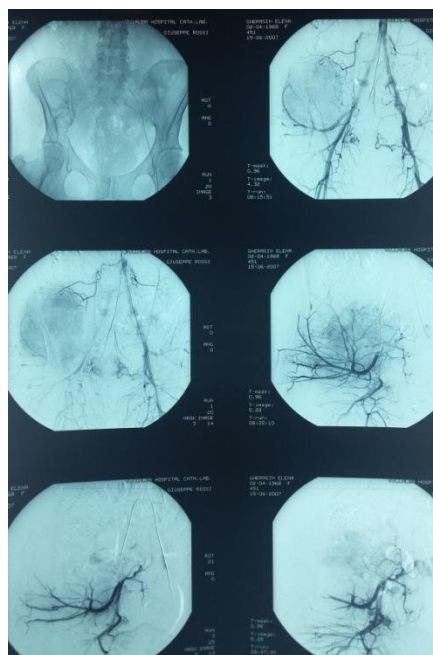


Fig 2 – Embolization using Glubran 2 gel



Fig. 3. Internal Hemipelvectomy type I+IIA Enneking

DISCUSSION

Oliveira et al. (1) state that with adequate physical therapy patients who undergo internal hemipelvectomy without further reconstruction may walk again after the pelvic fibrous plaque forms in about 1-3 months after surgery. Our patient did so 40 days after surgery. She showed no recurrences or complications, a satisfactory functional outcome and a favorable long-term prognosis, which demonstrated a pattern of myeloma evolution after internal hemipelvectomy similar to that of other types of malignancies reported in the literature (1, 2, 4, 5). The patient does not require any pain medication unlike the patients who undergo amputation, is in good psychological state and socially

integrated, results in agreement with previous studies (2, 4, 5).

CONCLUSIONS

In our patient, internal hemipelvectomy was the treatment option that spared the affected limb and avoided amputation, which is a traumatic radical alternative. Our particular case demonstrates a good functional recovery, regaining the ability to walk independently. The lack of complications and recurrences confirms the appropriate therapeutic selection. Indications for reconstruction should be reconsidered and individualized in order to achieve a long-term survival at the cost of weaker functionality.

REFERENCES

1. A.F. Oliveira, L.J. Vieira, A.C. Rodrigues do Nascimento, J.B. de Paula Fraga, R.R.L. Costa, L.G. Rodrigues do Nascimento. Internal hemipelvectomy: report on eight cases. *Rev Bras Orthop.* 2012; 47(6): 776-779.
2. A. Asavamongkolkul, R. Pimolsanti, S. Waikakul, P. Kiatseevee. Periacetabular limb salvage for malignant bone tumours. *J Orthop Surg.* 2005; 13(3): 273-279.
3. E. Hanganu. The multiple tumor syndrome. Personal experience. *Rev Med Chir Soc Med Nat Iasi.* 2002; 107(2): 338-342.
4. K.M. Robinson, R.D. Lackmann, R. Donthineni-Rao. Rehabilitation after Hemipelvectomy. *UPOJ.* 2001; 14: 61-69.
5. L.A. Beck, M.J. Einertson, M.H. Winemiller, R.W. DePompolo, K.M. Hoppe, F.F. Sim. Functional outcomes and quality of life after tumor-related hemipelvectomy. *Phys Ther.* 2008; 88(8): 916-927.

GIANT INTRALIGAMENTARY UTERINE LEIOMYOMA AND ITS COMPLICATIONS

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GIANT INTRALIGAMENTARY UTERINE LEIOMYOMA AND ITS COMPLICATIONS
(Abstract): Uterine leiomyomas are benign tumors arising from uterine smooth muscle. Although their pathogenesis remains unclear, they are the most common tumor of the female reproductive tract, occurring in as many as half of women older than 35 years. Uterine leiomyomas represent the most common benign tumors of the female reproductive tract. Giant uterine leiomyomas are very rare and represents a great diagnosis and therapeutic challenge. Uterine leiomyoma is one of the most frequent types of tumours and it is diagnosed in 20-40% of the women of reproductive age. Until the age of 50, approximately 70% of the white women and less than 80% of the black women will have had at least one leiomyoma. The frequency of the emergence of uterine leiomyoma in black women is significantly higher than in white women.

Keywords: UTERINE GIANT FIBROID, SURGERY, HYSTERECTOMY.

Around 200,000 hysterectomies, 30,000 myomectomies and thousands of selective uterine artery embolizations are performed annually in the United States to reduce the size of uterine leiomyomas (4, 5).

These lesions disturb the uterus functions and generate excessive bleeding, anemia, defective implantation of embryos, recurrent miscarriages, premature labour, pelvic discomfort and urinary incontinence; they can also imitate or mask malignant tumours.

The size of the leiomyoma and its location generate characteristic manifestations. Thus, in the case of submucous leiomyomas, there are excessive or irregular bleedings, infertility, as well as recurrent miscarriage. Subserous leiomyomas developing in the peritoneal cavity can exercise pressure, which is perceived by the patient as pelvic

discomfort, and intramural leiomyomas located in the myometrial wall are an intermediary grouping (7, 12).

CASE REPORT

Patient M.B., 44 years old, coming from the rural environment, working as a salesperson, is admitted in 2nd Obstetrics and Gynecology Clinic, at “Cuza Voda” Maternity Hospital of Iași for: moderate pain in the hypogastrium and left iliac fossa, pelvic load sensation, repeated metrorrhagias (excessive genital bleeding at irregular intervals, of prolonged duration of the flow > 6 days), enlarged abdomen, pollakiuria, slow bowel movement, pain in the left lower limb.

The personal pathologic history reveals that, 14 years before, the patient underwent surgery for left adnexectomy due to left

ovarian cyst, and her family history is insignificant. At the time of her admission, the patient's general state was good, and the examination of her apparatuses and systems reveals chronic constipation (slow bowel movement), and pollakiuria.

Upon bimanual abdominal palpation, in the left iliac fossa and hypogastrium an abdominal-pelvic tumour is found, which is hard, irregular, of firm consistency, painful upon palpation, of reduced mobility, and with the transversal diameter over 8 cm. The digital vaginal examination reveals a

cartilaginous cervix with the internal orifice closed, the uterine body enlarged on the whole, deviated to the left, of polycyclic outline, impalpable annexes.

The abdominal ultrasonography reveals a tumour formation that is hyperechogenic, non-homogeneous, well limited, of 24.4/31 cm, non-visualized annexes, no free fluids in the peritoneal cavity; in the left kidney, there is a 1st degree left hydronephrosis, due to the compression of the tumour. Laboratory tests reveal a mild anemia upon admission (fig. 1).

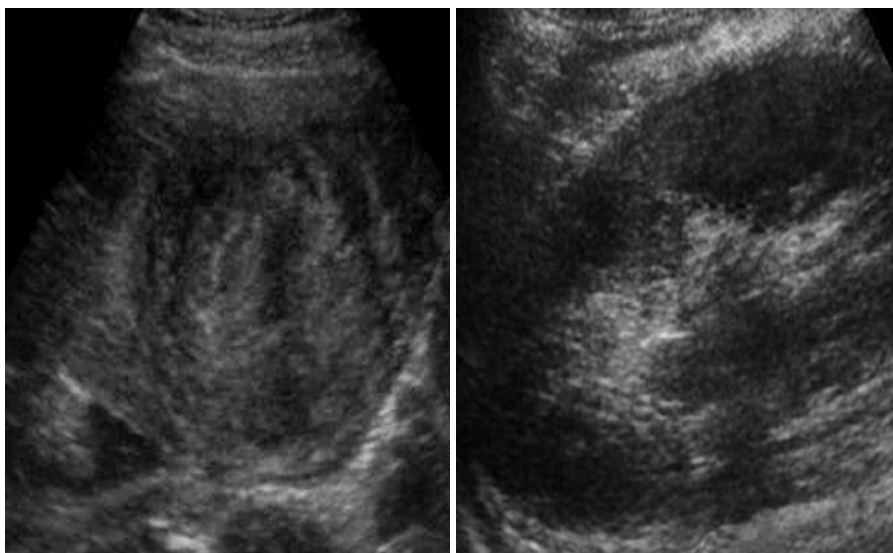


Fig. 1. Transabdominal ultrasound aspects of the uterine leiomyoma

Based on the clinical and paraclinical data, the set diagnosis is uterine polyfibromatosis with an intraligamentary nodule of 25/30 cm, for which surgical therapy is chosen. Surgery is performed and the following is found during surgery: enlarged uterus of approximately 11/9/5 cm, with multiple subserous fibromatous nodules with diameters between 3 and 5 cm, an adherent intraligamentary fibromatous nodule of approximately 25/30 cm, and a posterior

isthmus subserous nodule of approximately 7 cm, left annex absent by previous surgical intervention, right annex of normal aspect. Total hysterectomy and right adnexectomy are performed. Difficult hemostasis due to exuberant vascularization of the leiomyoma, but eventually efficient. Two intraabdominal meshes with hemostatic role are left inside as well as a drain in the Douglas space. Postoperative pieces were submitted to an anatomopathologic examination (fig. 2, 3, 4).



Fig. 2. Intraoperative image (uterine leiomyoma)



Fig. 3. Intraoperative aspects of the pelvic tumour

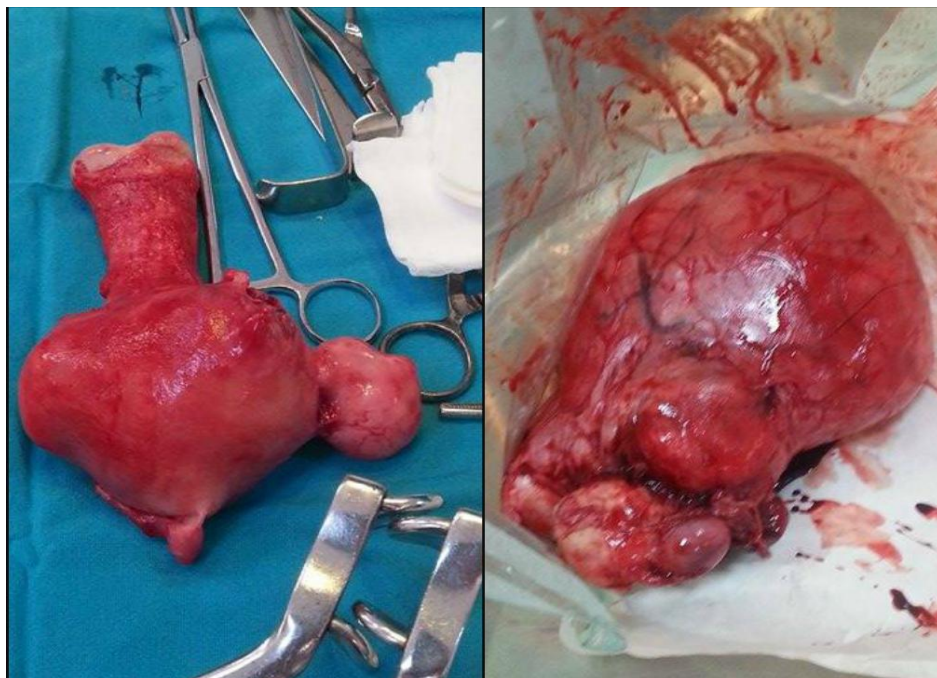


Fig. 4. Operative pieces (uterine body + cervix; intraligamentary uterine leiomyoma of 25/30 cm)

After surgery, following laboratory tests, the patient was transfused with two units of iso-group iso-Rh blood. Her evolution is favourable, she resumed feeding and bowel movement, the abdominal meshes and drain are suppressed the second day after surgery. A sample was collected from the wound, which was positive for *Staphylococcus* OR, sensitive to Ciprofloxacin.

In the anatomopathologic examination, the following is found in the examined sections: subserous nodular leiomyoma (max diameter 33 cm) with densely vascularized areas and hyaline degeneration with no mitotic activity, necrosis or haemorrhage, intramural nodular leiomyoma (diameter = 3cm), endometrium with simple hyperplasia and exacerbated chronic inflammation, non-specific chronic cervicitis, right ovary: follicular cysts, simple serous

cyst (2cm), right exacerbated chronic salpingitis.

DISCUSSIONS

Depending on the size and location, the uterine leiomyoma can put pressure on the uterine organs, generating a series of “borrowed” symptoms, of variable intensity, some forms being well tolerated, but others generating important complications.

Generally, leiomyoma is not painful, but in case pain emerges, then we can speak of a trophic complication of the tumour, compressive phenomena exercised on other organs and sometimes even an association to another pathology. Large tumours generate a load sensation in the pelvis, the submucous polypoid ones – spasmodic or colical pain, and in the case of enclosure of a leiomyoma in the true

pelvis, most frequently with isthmic location, there is pelvic pain radiating in the lower limbs due to the compression on the roots of the sciatic nerve. The latter phenomena appears in less than 1% of the cases and is favoured by pregnancy.

The leiomyoma location is important also in detecting other functional disorders, therefore in case of compression on the urinary ducts, there are urination disorders such as pollakiuria, which is generated due to the hindrance of the bladder distension by the leiomyoma. But the most important is the renal-urethral impact, which affects silently the upper urinary ducts, and in the case under discussion the leiomyoma – being intraligamentary – generates hydronephrosis, which comes to compromise the renal function. The posterior development of the leiomyoma can be accompanied by strangury or chronic constipation.

In the case of a leiomyoma with pelvi-abdominal development, the surgical intervention is more laborious, of high difficulty. Most times, the median incision is extended to the left paraumbilical area for increased visibility. The surgical option is determined most often by the tumour size, the organs need to be accurately recognized, avoiding the opening of the urinary bladder in the lower angle of the abdominal approach path. In order to avoid haemorrhage, the contact with the tumour surface should be as tight as possible.

In 1952, Forgue spoke about the precise notions of the detachable areas of the pelvic peritoneum, about the virtual subserous spaces where these tumours develop, and about the lesion risks involved by visceral displacements, which the surgeon should consider.

The surgical difficulty as regards a pelvic tumour of large size is not related to the

total size of the uterus, but rather to the presence of included leiomyomas, those tumours developed inside the areas of detachment of the visceral peritoneum.

The intraoperative problems are represented by the probable existence of adhesions between the uterus and neighbouring organs, the presence of intense vascularization feeding growing tumours with haemorrhagic risk. Several planes must be crossed in order to reach the true cleavage surface, these planes can include the ureters; the vessels feeding the tumour can be numerous and large, in some cases as big as the inferior vena cava. Thus, they need to be ligatured before acting in this area (1, 9).

Due to its location, the leiomyoma can displace the uterus, can elongate the vagina or even elevate the urinary bladder. This way, the relations to the organs around are changed, the main risk of the surgical intervention being injuries cause to the uterus. The advantage that the gynaecological surgeon has in case the tumour is benign is the fact that the uterus is displaced laterally by the tumour and not enclosed in it, as is the case of endometrial adenocarcinoma or cervical cancer, which invade one or both parameters from the very first stages. Therefore, we consider that procedures such as catheterizing the uterus before surgery or other imaging methods of evaluation of the exact location of the ureteral route are not absolutely necessary if the paraclinical evaluations prior to surgery support the diagnosis of benign uterine tumour. We consider that the attentive opening of the parameter with the evaluation “at sight” of the parameter elements and avoiding injuring them are the optimum procedure in such cases. The entire pelvic anatomy can be completely changed.

Hence, the surgical technique is not the

standardized one. The surgical intervention begins from the best exposed uterine side. At first, either adnexectomy is performed on that side, or the interadnexal hysterectomy technique is applied, followed by adnexectomy. Hysterectomy is performed concomitantly on both uterine sides, with the purpose of mobilizing the uterine body and exteriorizing it as much as possible. Another technique that can be performed consists in the initial sectioning of the uterosacral ligaments, which increases the uterus mobility. Total hysterectomy is also possible, but it involves higher blood loss and surgery takes longer. It would be optimum for the hysterectomy duration to be as short as possible and the blood loss to be as low as possible. This way, the surgical technique can be simplified by practicing first subtotal hysterectomy or polomyomec-tomy followed by the totalization of the intervention and potentially adnexectomy. The totalization of the intervention by extracting the cervix immediately after the subtotal hysterectomy is not subject to the same technical difficulties as totalization of a hysterectomy performed years before. In such cases, the visceral peritonization of the cervical stump determines the elevation of the urinary bladder, which is injured

during surgery, while uncovering the cervix. There is no such risk in our intervention (11, 12).

CONCLUSIONS

In our opinion, the therapy for the uterine leiomyoma must start from the particularities of this tumour: it is a benign tumour with negligible risk of turning into a malignant one, and the symptoms that the patient describes are given by afferent complications and not the leiomyoma itself, and it is when the uterine bleeding becomes abundant leading to anemia that it overcomes the stage of sign and it becomes a complication.

The entire pelvic anatomy can be completely changed. Hence, the surgical technique is not the standardized one. It would be optimum for the hysterectomy duration to be as short as possible and the blood loss to be as low as possible. This way, the surgical technique can be simplified by practicing first subtotal hysterectomy or polomyomec-tomy followed by the totalization of the intervention and potentially adnexectomy.

Also, the section of utero-sacral ligaments at the beginning of the hysterectomy is an efficient technique of making the uterus mobile.

REFERENCES

1. Crauciuc E, Niculescu Simona, Balan R, Amalinei Nela, Flaiser Corina, Butureanu St, Fibrom uterin gigant. Prezentare de caz. *Rev. Med. Chir. Soc. Med. Nat. Iasi* 2008; 112(1): 148-151.
2. Lefebvre G, Vilos G, Allaire C et al. The management of uterine leiomyomas. *J Obstet Gynaecol Can* 2003; 25 (5): 396-418.
3. Powell JL Giant fibroids. *J Am Coll Surg* 2004; 198(5): 844-845.
4. Downes E, Sikirica V, Gilabert-Estelles J, et al. The burden of uterine fibroids in five European countries. *Eur J Obstet Gynecol Reprod Biol* 2010; 152: 96-101.
5. Cramer SF, Patel A. The frequency of uterine leiomyomas. *Am J Clin Pathol* 1990; 94: 435.
6. Dragomir A, Schroeder JC, Connolly A et al. Potential risk factors associated with subtypes of uterine leiomyomata. *Reprod Sci* 2010; 17: 1029-1033.

7. Anania CA, Tewart EA, Quade BJ, et al. Expression of the fibroblast growth factor receptor in women with leiomyomas and anormal uterine bleeding. *Mol Hum Reprod* 1997; 3:685-689.
8. Robert HG. Nouveau traite de Technique Chirurgicale. Tome XIV. *Gynecologie*. Paris: Ed Masson, 1977; 264-273.
9. Lazarov N, Lazarov L. Giant uterus myoma in patient of 84 years old. *Akush Ginekol* 2007; 46(1): 48-49.
10. Thomas Ej. Add-back therapy for long-term use in dysfunctional uterine bleeding and uterine fibroids. *Br J Obstet Gynaecol* 1996; 103 Suppl 14:18.
11. Nguyen-Duc H. Large uterine fibroma in a 5 year old adolescent. Giant leiomyoma in adolescence. *J Gynecol Obstet Biol Reprod* 2003; 32 (8 Pt 1): 748-750.
12. Surcel IV. *Fibromioma uterin*, Ed Dacia, Cluj, 1990.
13. Hacker Moan. *Essentials of obstetrics and gynecology*, Saunders Elsevier, 1998.
14. American College of Obstetrics and Gynecologists. *Uterine Leiomyoma Educational Bulletin* No 192, Washington DC, ACOG, May 1994.

NEWS

THE IMPORTANCE OF MOLECULAR BIOLOGY TESTS IN THE DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE* INFECTION

C. difficile is an anaerobic bacterium that causes pseudomembranous colitis after antibiotic therapy. Between 3% and 7% of healthy people are colonized with *C. difficile*, but the percentage increases among people hospitalized. *C. difficile* infection diagnosis is based on clinical manifestation and is confirmed by the immunoassay test for *C. difficile* toxins and PCR for bacterial DNA. A recent study showed that the exclusive use of PCR in the diagnosis of *C. difficile* infection without immunological tests for toxins may conduct to over diagnosis and overtreatment. The duration of diarrhea during treatment, rate of complications and mortality rate for patients Tox-/PCR+ were comparable to patients without *C. difficile* (Tox-/PCR-) and lower than Tox+/PCR+ patients. These findings suggest that the treatment is not necessary for patients who were positive by PCR testing but negative by toxin immunoassay (Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, Nguyen HH, Huang B, Tang YW, Lee LW, Kim K, Taylor S, Romano PS, Panacek EA, Goodell PB, Solnick JV, Cohen SH. Overdiagnosis of *Clostridium difficile* infection in the molecular test era. *JAMA Intern Med*. 2015. doi:10.1001/jamainternmed.2015.4114).

Cătălina Luncă

DILEMMAS IN THE ATTITUDE TOWARDS SUICIDE

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DILEMMAS IN THE ATTITUDE TOWARDS SUICIDE (Abstract): Although apparently easy to define, the suicidal act or attempt raises complex and difficult problems due to the multitude of conditions and situations that can lead to it. In all cases the suicide's definition has always centred on the intention of one person to deliberately cause his or her death in an active manner. Defining suicide has been consecutively the temptation of philosophers, sociologists, theologians, psychologists and psychiatrists. From an epistemological point of view the suicide is an open concept without precise borders, yet not incoherent. Scientists have constantly tried to establish evaluation criteria of suicidal acts but these are variable. One can even assume that there is an infinity of combinations of characteristics that would legitimize the label of suicide, although none of them can be particularized in any way. Not even death itself represents a necessary condition for the evaluation of an act as suicide. **Key-words:** suicidal act, open concept, death by suicide.

Various attempts to define the suicide have been made in time by philosophers, sociologists, theologians, psychologists and psychiatrists. Sociologists, following the descriptive conclusions of E. Durkheim, regard suicide as the conscious assumption of a deadly risk (1). Certain psychiatrists, followers of Karl Menninger, are in striking opposition, believing that suicidal attitude is beyond mental integrity and consciousness. They state that any self-aggressive behaviour can be considered suicidal, and any lethal result of such behaviour can be qualified as suicide. In his attempt to come as closely as possible to an accurate definition of suicide Tom Beauchamp established three evaluation criteria for any suicidal act: the individual intentionally causes his/her death, there is no coercion in this respect from any other

individuals and death is caused by circumstances especially created for the suicidal act purpose (2). Regardless of the accuracy of these criteria used in the identification and evaluation of the suicidal act, such a complex phenomenon cannot be comprised in a single succinct definition. It can occur in very different circumstances and can be performed through various methods. Motivation is often vague, mysterious and incomprehensible for both specialists and people who are close to the person who commits suicide.

From an epistemological perspective suicide appears as an open concept, without precise borders, without lacking coherence. There have been some attempts to establish evaluation criteria for a certain act in order to classify it as suicide, but these are variable. One could legitimately assert that there

is an infinity of combinations of characteristics that would justify labelling an action as suicide, yet they cannot be particularized, as not even death itself represents a necessary condition for evaluating an action as suicide (3).

Consequently, although it is apparently easy to define the suicide or suicide attempt, it still raises plenty of complex and difficult issues, due to the multitude of conditions and situations it can occur in. In both reductionist approaches and more liberal perspectives the definition of suicide focused on the intention of that individual to deliberately cause his/her death, in an active manner (4). The ones in favour of the reductionist approach were more radical and emphasized the self-destructive intention, establishing at the same time the purpose and the obvious motivation of the intention and the action. There is a very short distance from defining suicide in such a way to becoming judgemental; judgement requires pros and cons, leading to the paradox that the evaluation of the motivation is taken into account more than the existence of a desire to die. Many times the intention can be caused by the desire to change a certain state of things, which actually distances this act from suicide proper. The examples of people who commit suicide in order to end the pain in terminal illnesses such as cancer or of those who want to avoid betrayal during wartime are quite illustrative for the complexity of the issue. In these circumstances, suicide cannot be defined without a complex analysis of its dimensions as intention, deliberate act and consequences.

PHILOSOPHICAL AND MORAL CONSIDERATIONS

For thousands of years, religious tradi-

tions have imposed a very peremptory attitude on societies towards suicide. In India, the Upanishads tradition recognizes the suicidal act as a decent, sometimes heroic way of dying, yet only by drowning, fire or starvation. Later on, Hinduism and Jainism accepted and allowed passive self-destruction through starvation and asceticism. This form of passive self-destruction dedicated to certain deities is still opposed to certain active suicide methods, actually prohibited by the Hindu religion. In time, Buddhism adopted a very clear attitude towards any form of self-destruction. Escaping from a world of suffering was not allowed by means of suicide, the alternative being a change of attitude towards the self and the world. In the history of the Far East, two self-destructive modalities, in other words two motivations of the suicidal act were allowed, namely honour and revenge.

In ancient and medieval China, a man's suicide was disapproved because the man's duty was to support his family. Women did not have the same duty, on the contrary, they were often forced to defend their honour by suicide. As well as this, in both India and China, the cremation of the wife on a funeral pyre, together with the deceased husband was a common practice, that persisted for many years. As long as the wife expressed her consent, this practice could be regarded as an act of suicide, accepted and even imposed by the society.

The European medieval religious tradition adopted an exceptional attitude according to which death is preferable to the betrayal of God. Thus, imposed apostasy represented the cause of many admitted suicides in both the Christian and the Judaic religions. The Islam, another monotheist religion, forbids suicide on the grounds of

God's supreme right upon any life. Consequently, the Islamic tradition forbids, through its Prophet's words, included in the Sunnah, the burial of the one whose death is caused by suicide, the Koran stating that "no soul is allowed to die unless God allows it at a certain moment".

The idea of gods' sovereignty upon human life is also to be found in the Greek philosopher's writings. The personal authority on someone's own life is limited by the priority of gods' sovereignty, suicide being regarded thus as an usurpation of divine power (5).

The same arguments were brought forward in medieval Christianity. Thomas Aquinas claimed that suicide represented an offense against one's own person, against the society and especially against God, since man belonged to his creator, who had offered him the gift of life. This concept of gift of God represented for many contemporary theologians the basic argument in their opposing the suicidal act. This opposition has not always been unanimously embraced. Thus, David Hume, in his essay *On Suicide*, claims that "God does not immediately appear in any activity, yet He governs everything by those general and immutable laws that have been established from the very beginning". Consequently, suicide may be part of the pre-established destiny of every human being, being regarded any other death cause (6).

We should nevertheless point out the differences between religious viewpoints and those based on moral grounds. Starting from various theories of morals, the Stoics or the Romantics of the 19th century displayed an attitude opposed to that of the church, looking for socially rooted arguments. This way, the attitude towards suicide was less radical during the 20th centu-

ry. Even Theology raised the question whether suicide represents an act of self-sacrifice or self-suppression. The sinful dimension of suicide regarded as a crime against one's self is gradually lost; especially when related to medical issues, suicide becomes a more complex problem that transcends theological, philosophical or sociological areas (7).

The media mentions suicide cases very often all over the world. One argument against those who commit suicide is most of the times obvious and it seems to be shared by most people. The suicidal act is judged as cowardice in front of the hardships of life. Landsberg's objection is that "Hannibal's or Seneca's ways of dying could hardly be regarded as acts of cowardice". He also adds that "there are certainly more people who do not commit suicide because they are too coward to do it than people who commit suicide because they are coward". This does neither imply that Landsberg views suicide as act of heroism or that he justifies suicide for a noble purpose. He simply shows that the suicidal act is not merely a cowardly action, as most often labelled (8).

For Cioran, the absurdity of life is what causes a feeling of repulsion towards it. Moreover, he considers that the pursuit of truth is a battle in vain, as death is the only certainty of life. Starting from these premises, Cioran develops, in an almost Schopenhauerian manner, an ethics of renouncement. Unlike Schopenhauer, where the tragedy is more profound, as will is present in absolutely everything, influencing both the phenomenological and the intelligible worlds, Cioran still hopes that life is the only refuge. From this perspective, he states: "There are no arguments for living. On the heights of despair, the pas-

sion for the absurd is the only thing that can still throw a demonic light on chaos". It becomes evident, that under the dominance of the absurd, life loses its meaning. In these cases, suicide remains, for existentialist thinkers, a valid solution. The issue is no longer considered from a moral perspective, as Cioran himself states: "here we must think beyond normative conventions, as the issues addresses the human condition directly" (9).

CLINICAL FORMS OF SUICIDE

The suicide is probably the most dramatic and challenging clinical situation a psychiatrist is confronted with. It does not only represent a functional problem that could be addressed by means of therapeutic techniques, but also an existential issue, in both the proper and the philosophical meanings of the term (10, 11, 12). It is not about getting to lead a better life but about life being worth living or not. The fact that the starting point in the treatment of a potentially suicidal patient is seen in such different terms by the therapist and the patient qualifies the suicide as an extremely difficult case, since the psychiatrist duty goes beyond helping his/her patient by technical means. More precisely, the former is supposed to persuade the patient to alter his/her most profound beliefs and attitudes concerning life.

From a clinical viewpoint, the suicidal phenomenon displays numerous individual variations, yet some common traits have been identified (13):

- the purpose envisaged by suicide is the need to find a solution
- the target to be achieved is suspending the state of consciousness, stopping the flow of consciousness
- the triggering stimulus is uncon-

trollable psychological pain

- frustrate the common stressor in suicide is represented by frustrated psychological needs
- the person who commits suicide experiences an overwhelming feeling of helplessness and despair
- the inner attitude leading to autolytic actions is ambivalence, that is experiencing two contrary tendencies: of self-destruction and of planning a rescue
- the cognitive status of suicidal people is constriction, a major narrowing of the cognitive field and of the capacity to produce response alternatives
- the common action in suicide is escaping, avoidance to confront certain situations perceived as stressful (13, 14)

G. Ionescu enumerates the following forms of suicide: the idea of suicide, the suicide attempt, dissimulated suicide, suicidal drive and chronic suicide (15).

- **The idea of suicide** is not necessarily pathological in nature as it can also appear in the larger area of normality. It is probably exacerbated in crisis situations, when the individual cannot access a response method that s/he considers satisfactory.
- **The suicide attempt** is a failed suicidal act. As far as their truthfulness is concerned, suicide attempts could be placed on a bipolar axis, having as extremes full authenticity and provability.
- **Dissimulated suicide** often surprises due to the preoccupation of the subject for the feelings and wellbeing of the people around

him/her, especially those of the family members. The subject appeals to dissimulation in order to make sure s/he does not impose too heavy a moral and social burden on the loved ones, who are most often likely to experience self-culpability feelings.

- **Suicidal drive** explains the suicidal act as an expression of an irresistible, impossible to repress tendency of the individual to end his/her own life. Like any drive, the suicidal drive is transient so that the individual, once saved, is surprised by his own gesture.
- **Chronic suicide** includes the following situations:
 - risk behaviours taken to extremes to aberrant behaviours
 - refusal for absolutely necessary surgical interventions
 - refusal of absolutely necessary medical treatment
 - continuing to take drugs or drink alcohol in cases when the physical, psychic and professional degradation of the person is obvious

As opposed to various other types of psychological disorders, the suicidal state is not one that could gradually improve or worsen, but it represents a radical event that occurs at one given moment, is totally unpredictable and often irreversible.

ETHICAL ASPECTS

For the psychiatrist the ethics of suicide centres firstly upon the moral justification and the limits of an intervention policy. Thomas Szasz is known for his vehement attack against the traditional psychiatric approach of suicide, claiming that “accom-

plished suicide is generally the expression of an individual desire for greater autonomy and particularly for self-control upon one’s own death”. He considers that intervention in the suicidal behaviour is always inappropriate, actually diminishing the individual’s freedom and reflecting the coercive patronizing attitude of the therapist (16).

Suicide faces thus the psychiatrist with a dilemma from both a professional and a human point of view. The therapist is challenged by the need to choose between saving a person’s life, as stated in the Hippocratic Oath, and his duty to respect a personal decision, according to the ethical principle of individual autonomy (17). The critical moment extends to the point where the psychiatrist’s right to interfere becomes the direct consequence of establishing an individual’s ability or inability to decide upon his/her own life. Regardless of all these dilemmas, suicide prevention remains a desiderate and a reality at the same time, being regulated by national programmes adopted even by the most liberal countries as far as an attitude towards individual freedom is concerned (18).

The utilitarianism and other philosophical theories are not able to provide all necessary arguments for evaluating the value of life, as, from a moral viewpoint, life should be respected for its intrinsic value and consequently there might appear divergences of views between the psychiatrist and his/her potentially suicidal patient. The psychiatrist justifies his/her intervention pleading for the value of life, while the patient can quote arguments related to the quality of his/her life, disregarding the value of life in itself. The difficult task of the psychiatrist is reflected in his/her attitude that should replace the paternalist

approach with persuasion. He/she should make the patient perceive the new meanings his/her life might gain, allowing him/her at the same time to be completely free in making a decision.

CONCLUSIONS

All rational arguments in favour of suicide, such as avoiding pain or dishonour, actually plead in favour of giving up a certain quality of life, without actually inquiring whether death is better than life. The images of both death itself and life in its intrinsic dimension are equally difficult to perceive. Even psychiatric pathology can offer countless examples of delirium-hallucinating states related to experiences and identities that occur after death. Regarded from a life's value perspective,

suicide cannot have a specifically moral representation, as morality implies a series of rules and principles that are the very grounds of life. Suicide ends up thus representing a decision beyond the sphere of moral arguments and other people's judgements. Consequently, the reason for which suicide should be prevented starts from the value of life in itself, and the medical intervention in the suicidal act is justified by the very belief in the value of life.

Suicide prevention is unfortunately still a mere palliative method, all positive results having just an individual character, while suicide rates remain, except few variations, quite constant. Suicide remains thus a ubiquitous, universal and perennial phenomenon, whose social impact and resonance cannot be neglected.

REFERENCES

1. Durkheim E. *Despre sinucidere*. Ed. Institutul European, 1993.
2. Beauchamp TL, Seymour P. *Ethical Issues in Death and Dying*. Prentice Hall, 1978.
3. Scripcaru C, Chiriță R, Scripcaru Gh. Conduite suicidaire: epistemologie medico-legale. *Revista Medico-Chirurgicală* 1993, 2: 197-203.
4. Vedrinne J. *Suicide et conduites suicidaires*. Masson, Paris, 1981.
5. Farberow NL. *Suicide in Different Cultures*. University Park Press, Baltimore, 1975.
6. Hume D. *On suicide. Essays*. Longman, London, 1882.
7. Narveson J. Moral philosophy and suicide. *Canadian J Psych* 1986, 31: 104-107.
8. Landsberg PL. *Problema morală a sinuciderii*. Humanitas, București, 1992.
9. Cioran E. *Pe culmile disperării*. Humanitas, București, 1990.
10. Brânzei P. *Itinerar psihiatric*. Junimea, Iași, 1979.
11. Pirozynski T. *Psihiatria și condiția umană*. Psihomnia, Iași, 1995.
12. Chiriță V, Papari A. *Tratat de psihiatrie*. Ed. Fundației „Andrei Șaguna”, Constanța, 2002.
13. Cosman Doina *Psihologie medicală*. Polirom, 2010.
14. Barraclough B, Bunch L, Nelson B, Sainsbury B. A hundred cases of suicide: clinical aspects. *Brit J Psych* 1974, 125: 355-373.
15. Ionescu G. *Tratat de psihologie medicală și psihoterapie*. Asklepios, București, 1995.
16. Szasz T. The case against suicide prevention. *Am Psych* 1986, 41: 806-812.
17. Chiriță V, Chiriță R. Conceptul etic în psihiatrie și calitatea vieții. *Revista Română de Sănătate Mintală* 1998, 2: 18-21.
18. Dew MA, Bromet EJ, Brent D, Greenhouse JB. A quantitative literature review of the effectiveness of suicide prevention centers. *J Consult Clin Psych* 1987, 55: 239-244.

REDUCE CHILD MORTALITY AS A MILLENNIUM DEVELOPMENT GOAL IN ROMANIA

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REDUCE CHILD MORTALITY AS A MILLENNIUM DEVELOPMENT GOAL IN ROMANIA (Abstract). **Aim:** To assess the efforts made in Romania towards achieving the Goal 4 from MDGs - Reduce Child Mortality. **Materials and methods:** A descriptive study about the deaths among Romanian children under five, between 2002 and 2015, from the perspective of the MDGs. To help track progress toward this commitment, following specific targets and indicators were developed: Target 1- Halve the mortality rate in children aged 1-4 years between 2002-2015; Target 2 - Reduce infant mortality by 40% between 2002 and 2015; Target 3- Eliminate measles by 2007. The comparison allows establish the status (achieved or not) for each target. **Results:** From 2002, the under-five mortality rate recorded a continuous descendent trend till now (20.8 to 10.3 under five deaths per 1000 inhabitants in 2013). The infant mortality rates declined from 17.3 to 8.5 deaths per 1,000 live births (2002-2013). Eliminating measles by 2007 - was achieved one year later, because of the measles epidemic in 2005 and 2006. High vaccination rates have been maintained, with the proportion of children 1 year old vaccinated against measles reaching and being maintained at between 94-98%. **Conclusions:** Substantial progress has been made in Romania, in achieving the Millennium Development Goal no. 4. All the three targets were achieved. However, infant mortality still remains above the average of European Union (4 infant deaths per 1,000 live-births). **Keywords:** MILLENNIUM DEVELOPMENT GOALS, UNDER-FIVE MORTALITY, INFANT MORTALITY, ROMANIA.

In September 2000, 189 countries from the total of 193 member states within the United Nations signed the Millennium Declaration, with the scope to eradicate the extreme poverty in all its forms by 2015. The commitment was based on a set of time-bound and quantified goals and targets, called the Millennium Development Goals, focused on combating poverty in its many dimensions - including reducing

income poverty, hunger, disease, environmental degradation and gender discrimination (1,2).

These goals were created to improve the health status and wellbeing of people worldwide.

The Millennium Development Goals (MDGs) include 8 goals, 21 targets and 60 indicators for measuring progress between 1990 and 2015, when the goals are ex-

pected to be met (3):

- Goal No.1 – Reduce Severe Poverty
- Goal No.2 – Increase Compulsory Education Graduation Rate by 2012
- Goal No.3 – Promote Gender Equality and Empower Women
- Goal No.4 – Reduce Child Mortality
- Goal No.5 – Improve Maternal Health
- Goal No.6 – Combating HIV/AIDS, Tuberculosis and Other Diseases
- Goal No.7 – Ensure Environmental Sustainability
- Goal No.8 – Develop Global Partnerships for Development

The target in the Goal No.4 was “*Reduce by two-thirds, between 1990 and 2015, the under-five mortality rate*” and the included indicators were:

4.1 Under-five mortality rate;

4.2 Infant mortality rate;

4.3 Proportion of 1-year old children immunized against measles.

The use of infant mortality rates as a measure of overall health status for a given population is based on the assumption that it is particularly sensitive to socio-economic changes and to health-care interventions (4).

The infant mortality rate is commonly used as an indicator of the level of health in a community. The vast majority of these deaths are preventable with current technology, as evidenced by the extremely low child mortality risks prevailing in rich countries.

The purpose of this article is to assess the efforts made in Romania towards achieving the Goal 4 from MDGs - Reduce Child Mortality.

MATERIALS AND METHODS

This research represents a descriptive study about the deaths among Romanian

children under five, between 2002 and 2015, from the perspective of the MDGs.

In the United Nations Millennium Campaign, Romania committed herself to reduce child mortality (5). To help track progress toward this commitment, following specific targets and indicators were developed:

Target 1: Halve the mortality rate in children aged 1-4 years between 2002-2015;

Indicator: Mortality rate in children aged 1-4 years;

Target 2: Reduce infant mortality by 40% between 2002 and 2015;

Indicator: Infant mortality rate;

Target 3: Eliminate measles by 2007;

Indicator: Proportion of 1-year-old children immunized against measles.

The comparison allows establish the status (achieved or not) for each target. The data sources were represented by the National Institute of Statistics, yearbooks of the Ministry of Health and Romanian Government.

RESULTS

Target 1

The risk of a Romanian child not reaching his or her fifth birthday was nearly six times greater in 1970 (66 deaths per 1000 inhabitants) than nowadays (6,7).

Under five years of age mortality rate recorded in 1990 a still high level of 38 children aged 1-4 per 1000 inhabitants. Between 1990-2000, under-five deaths overall declined to 22.2 per 1000 inhabitants. From 2002, as specified in target 1, the indicator recorded a continuous descendent trend till now (20.8 to 10.3 under five deaths per 1000 inhabitants in 2013), as presented in fig.1 (8).

Target 2

The infant mortality rate is the second

of three indicators used to monitor achievements towards the Fourth Goal of the eight Millennium Development Goals. In the last four decades (1970-2010), the infant mortality rates have significantly declined.

From a high of 49.4 deaths per 1,000 live-births in 1970, infant mortality has dropped to 29.3 in 1980, respectively 26.9 in 1990 (32 deaths per 1,000 live births in rural areas). In 2000 it was 18.6 and subsequently, declined at 9.8 (2010). Throughout the current decade is 8.5 deaths per 1,000 live-births (2013). The evolution of the infant deaths between 2002 and 2015, as specified in the target 2, is described in fig. 2.

Since year 2000, the infant mortality pattern has changed, the perinatal conditions replacing the respiratory diseases as a first cause of infant death. In 2013 the leading causes of death among children under one year were:

- perinatal conditions (38.8%);

- respiratory diseases (23.5%);
- congenital anomalies (23.5%).

Infant death may occur in neonatal or post-neonatal period. In Romania, the ratio between neonatal and post-neonatal deaths changed, in favor of the first. Thus, in 1990 the values for neonatal and post-neonatal rates were 8.9 and respectively, 18 deaths per 1,000 live births, whereas in 2012 they declined to 4.8 and respectively, 4.2 (8).

Target 3

The target referring to eliminating measles by 2007 was reached later, in 2008, because of the measles epidemic in 2005 and 2006. However, the number of vaccinations has been significantly increased, the epidemic has been controlled and in 2008 and 2009 no new cases of measles have been reported. High vaccination rates have been maintained, with the proportion of children 1 year old vaccinated against measles reaching and being maintained at between 94-98% (9).

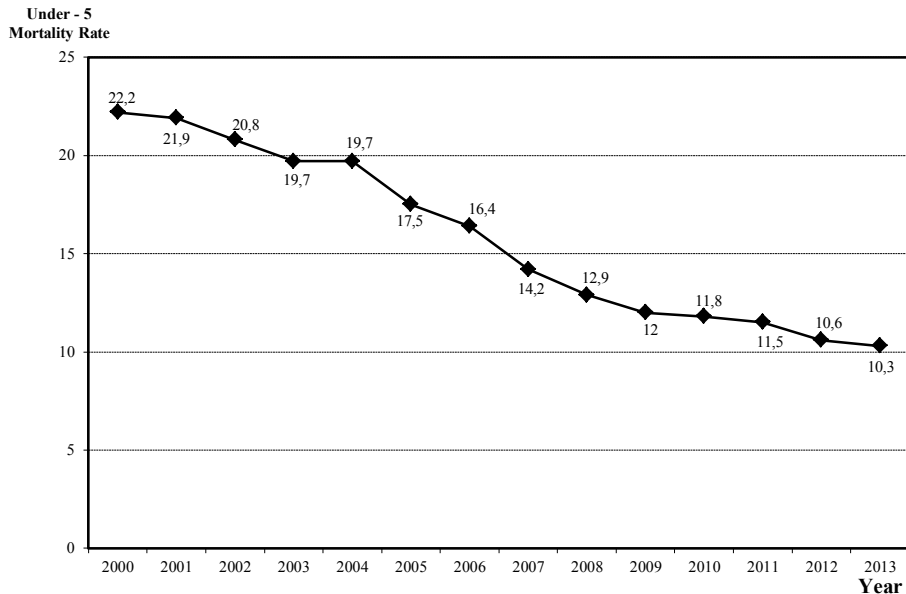


Fig. 1. Evolution of Under-five mortality rate in Romania, 2000-2013
(Romanian Statistics Annuary, 2014)

DISCUSSION

In Romania, mortality of children aged between 1 and 4 years dropped between 2002 and 2013 by 47.8%. The national target of halving the indicator (2002-2015) was achieved.

The United Nations worldwide target of a two-thirds reduction in under-five mortality (1990-2015) has already been achieved

since 2009. Annual rate of reduction (1990-2013) was 5%.

Large reductions in infant mortality rates have occurred in Romania. The second target of the Millennium Development Goal no. 4 was achieved in 2012, but the indicator still remains well above the average of European Union (4 infant deaths per 1,000 live-births).

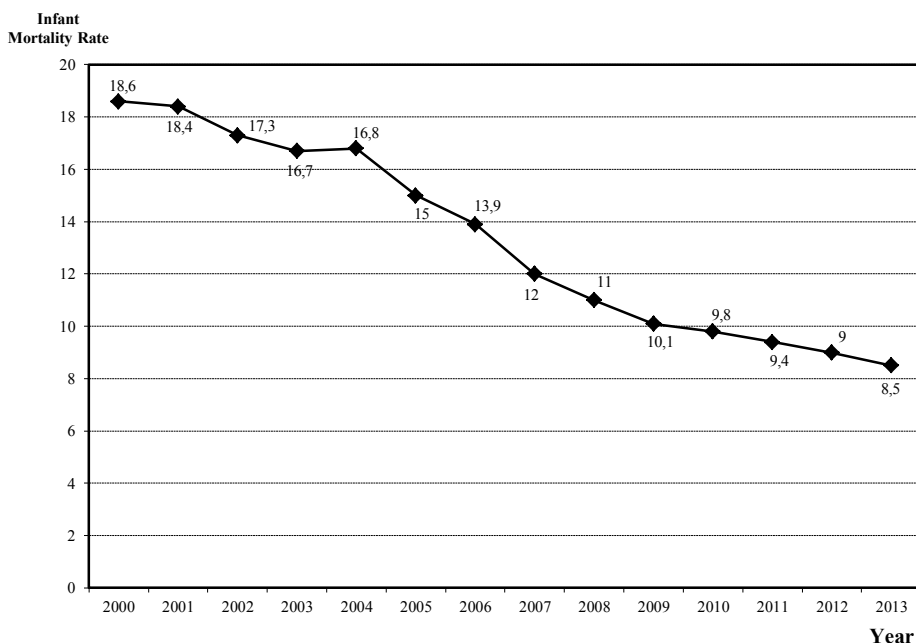


Fig. 2. Evolution of infant mortality rate in Romania, 2000-2013
(Romanian Statistics Annuary, 2014)

Neonatal mortality represents a significant proportion of child mortality (approximately 45%) and the child health Goal will not be reached unless neonatal mortality is addressed. The causes of neonatal death are different from the causes of death among older children. Health child programs and policies have to be focused on reducing neonatal mortality.

In order to maintain in the future the control of measles (target no. 3), the proportion

of one-year-old children immunized against this disease must be kept within $96 \pm 2\%$

CONCLUSIONS

Substantial progress has been made in Romania, in achieving the Millennium Development Goal no. 4 - reducing child deaths - since 1990.

The global target no. 1 to reduce by two-thirds, between 1990 and 2015, the under-five mortality rate was achieved. The

national target no.1 - to halve mortality of children aged between 1 and 4 between 2002 and 2015 - was also, achieved.

The target no. 2 - Reduce infant mortality by 40% between 2002 and 2015 - was achieved. However, with regard to both indicators concerning child mortality, in

spite of the remarkable progress made, Romania still lags far behind the other countries of European Union.

The target no. 3 - eliminate measles by 2007 - was achieved one year later (2008). These important gains will have to be maintained in the future.

REFERENCES

1. *** The Millennium Development Goals Report 2015. United Nations, 2015.
2. Toader E. Ethics in medical technology education. *Romanian J of Bioethics* 2010; 8(2): 157-162.
3. *** *Millenium Project* [Internet]. USA: Goals, targets and indicators, c2015 [cited 2015 Jul 5]. Available from: <http://www.unmillenniumproject.org/goals/>
4. Cărăușu EM. Le Management de la Santé. Travaux pratiques pour les etudiants de la Faculté de Médecine Dentaire. Bucharest: Maison d'Edition "Carol Davila", 2015.
5. *** *Raport asupra Obiectivelor de Dezvoltare ale Mileniului*, elaborat de Guvernul României cu sprijinul Sistemului ONU în România. București, 2003.
6. *** *Anuarul Statistic al României 2013*. Institutul Național de Statistică, 2014.
7. *** World Health Statistics 2015. Geneva: WHO, 2015.
8. *** *Anuar de statistică sanitară 2013*. Centrul Național pentru Organizarea și Asigurarea Sistemului Informațional și Informatic în Domeniul Sănătății. București: Ministerul Sănătății, 2014.
9. *** *Raport de activitate pentru anul 2012*. Guvernul României, Ministerul Sănătății, 2013.

NEWS

INTERNAL HERNIAS AFTER BARIATRIC SURGERY

Hernias are among the most common long-term complications after bariatric surgery. Incisional hernias occur very often after conventional open bariatric surgery. The incidence of internal hernias after bariatric surgery (a special challenge) is more common than for other upper gastrointestinal tract operations. These hernias are not limited to laparoscopic procedures but are also observed after conventional open gastric bypass surgery. As the incidence is significantly increased after laparoscopic interventions, there seems to be a close association with minimally invasive procedures. The clinical symptoms of internal hernias without complete obstruction are non-specific and the correct diagnosis is often not straightforward. In addition to the aspects of prevention of internal hernias in laparoscopic surgery, this article discusses the diagnosis and treatment, taking into account the various forms of internal hernia after bariatric operations (Karcz W et al. Internal hernias after bariatric surgery. *Der Chirurg* 29.08.2015 86 : 9 : 855-860).

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HEALTH CARE ECONOMICS IN ROMANIA – DYNAMICS AND EVOLUTION

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HEALTH CARE ECONOMICS IN ROMANIA – DYNAMICS AND EVOLUTION (Abstract): Health economics refers to the analysis of medical institutions considering their economic and social efficacy, but also the regularity and the relationships that govern the phenomena and the processes from the field of health with the final purpose of achieving better results with the minimum of resources; it represents the study of health price in its complexity. The economics of the population's health needs and in particular the health needs in case of the poor groups of the population, consider health to be the main component of global human vulnerability. Health economics tries to change the simple interpretation of health price and disease cost into a wider consideration of a system administration similar to educational and social economics and the study of health in the context of the multiple specializations of the macro economy of the national group, as it is an instrument in the country's great economics symphony. **Keywords:** MANAGEMENT, HEALTH ECONOMICS, HOSPITAL SERVICES.

Health economics appeared as a result of the interest the economists showed in all social life's fields, starting with industry and agriculture and finishing with education and health.

In general, it is considered that the population's state of health deteriorates and the mortality increases when the macroeconomic situation gets worse. Nonetheless, nowadays, there are more and more data indicating the fact that physical health and not psychological health, actually improves in this type of periods (1).

In the middle of an age of interdisciplinarity, economists see health as an economic good. It is determined by biological factors (heredity, sex, and age), environ-

mental factors and life style factors. But since prevention and early identification of diseases can be accomplished with huge financial investments and because health is quantified in terms of efficiency and efficacy, it becomes an object of economic study.

This is only one of the arguments that place health in its position of segment of the national economy. Consequently, the economists wander to what extent it answers to the supply and demand relationship, what the society wants, what it is offered to it and what it gives in return. The answers are difficult and relative because health, just like education, provides long-term services. But precisely this type of

observations confirms the efficacy and social utility of the health care system. Naturally invaded by numerous and different diseases, in its need of preservation, the society formulates requests that cannot be postponed.

Medicine represents a dynamic field if we consider the technology it works with, but completely inert if we refer to its financial situation, as the reform of the health care system requires time and the answers to macroeconomic shocks take numerous years, even decades. Even if the population presents a better state of health which generates higher incomes and vice-versa, the improvement of life standards leads to a better state of health in people, the relationships between the state of health and economics remain difficult to quantify and even study (2).

As an answer, the health care system comes with a diverse offer of services. The national level and its economic fund generate the value a society invests in health. Human value health is a right of man protected by medical science but also by economic resources and means.

ETHICS AND HEALTH CARE

Decades ago the differences between health economic analyses and clinical practice were significant, with the physicians and patients who defended Hippocrates' Oath on one side and policy makers and health economists on the other side. It was the policy makers the first ones to have to solve the difficult allocation problem: the health care budget was growing faster than any other public spending. Later, the economists came in trying to make the optimal decisions in limited financial situations. Then, health policy makers decentralized their budget, which was an intelligent political move, as the responsibility for the

healthcare budgetary allocations was now shared. However, clinical quality concerns started to appear: waiting lists and insufficient care, these rapidly becoming hot subjects in the public eye, with patients claiming their right for health care by law or by public indignation. It became clear that budget policy had to be justified by more arguments than just financial constraints.

In this context, the health's base results to be economic, and its management consist in obtaining maximum performances with the minimum (often limited) investments. This is how health economics was born, a science that can be mastered only by those with a dynamic, flexible thinking manner and endowed by the ethics of the health care system (5).

Ethical rationale for publicly financed health insurance rely on four logically related issues: (1) the ultimate purpose of a human life or human society; (2) the role of health and its distribution in society in advancing this ultimate purpose; (3) the role of access to or utilization of health care in maintaining or improving the desired level and distribution of health among members of society (4) the role of public financing in ensuring the ethically justified access to and utilization of health care by members of society.

It is true that economics must not prevail over the medical act, but it is equally true that the physician cannot ignore the efficacy of his actions (maximum results with minimum of resources) and their efficiency (health days, recoveries, years of life regained, lives saved). Deriving from political economics, health economics is part of a triad, along with education economics (medical education, in particular) and science economics (medical scientific research).

All the three entities work together un-

der the auspices of economics, according to the law based on maximum efficacy with the minimum of effort. The collaboration between physicians and economists results a necessity that can only be fulfilled through interdisciplinarity. Health economics contributes to economic health (6). C. Gastmans (cit. by 6) pleads for economists and ethicists to try and approach each other so that the tool of cost-effectiveness analysis is further developed to combine the two most important goals of health care systems: health maximization and the reduction of health inequalities.

In Romania, because the allocation of resources does not yet represent a purpose in itself, this necessity (for health economists) is less present. Nonetheless, there is hope for a rational perspective that might, at least support, if not impose, a decision and a smooth regulation of the decisional process.

HEALTH CARE ECONOMICS AND HTA

Romania spends approximately 6 percent of GDP (*gross domestic product*) on health (public and private) vs. the EU average (all EU countries) of 9.8 percent. Among current challenges are lagged health outcomes, patient dissatisfaction, lack of access to quality care by the poor and other vulnerable groups, and poor financial performance.

Romania had the highest infant mortality rate in the EU (3). Furthermore, the life expectancy gap between Romania and EU15 (EU countries on December 31, 2003) has increased 2 times since 1970, mainly due to the rising incidence of non-communicable diseases (NCDs).

Romania's health infrastructure and its service delivery system have not adjusted to modern technologies and do not meet the

current needs of the population, which has suffered both a demographic and an epidemiological transition in last two decades. The hospital network in Romania is large and fragmented, with over 350 public hospitals that have been mostly been decentralized. Except for SMURD (*Mobile Emergency Service for Resuscitation and Extrication*) and several maternities, no other significant investments have been made in the last 20 years in medical infrastructure.

The rhythm of health care sector reform has been slow due to the lack of financial resources required to support the new policies.

Health technology assessment (HTA) is the 'systematic evaluation of medical technologies regarding their effectiveness, appropriateness, efficiency as well as social and ethical aspects and implications'(1). Its main purpose is to inform technology-related policymakers in health care and thus has a decision-support role in pricing and reimbursement. HTA provides the responsible bodies and organizations with timely, accurate, and sound information on new medical technologies (3, 4).

HTA focuses primarily on pharmaceuticals and medical devices (standardized technologies with clear ownership) as these constitute a high financial cost and their social impact and visibility are significant (7).

As pharmaceutical expenditures grew rapidly over the past decade and the 2008 crisis posed a major financial risk, the Romanian government, financed by the World Bank, contracted as consultants NICE International (*The National Institute for Health and Care Excellence*) (UK) to provide recommendations on how to reform the health care system. The legal framework for HTA in Romania was created

through Government Decision 351/2012. The responsibility of the HTA unit is broad: HTA can be applied to all existing medical technologies such as pharmaceuticals, medical devices, health policies and public health.

According to an HTA guideline, the assessment of innovative drugs is made using a 6-item scoring chart where the maximum number of points is 10, and for a positive reimbursement recommendation, the pharmaceutical product has to score at least 6.

The decision on reimbursement is reached by assessing two distinct types of evidence:

1. Reimbursement recommendations given by HAS (Haute Autorité de Santé) France, and 3 HTA bodies in the UK (NICE - *The National Institute for Health and Care Excellence* / SMC - *Scottish Medicines Consortium* / AWMSG - *All Wales Medicines Strategy Group*), and reimbursement status in other EU member states;

2. Clinical profile of the intervention: relative efficacy, relative safety and *patient reported outcome* (PRO).

The family physician, being well informed about the social and demographic dimensions of health care and on the extremes of medical consumption because he deals with patients of all ages, must be aware of the balance between promoting health and treating diseases, the amount of services provided, their cost and efficacy.

Applying the cost-advantage and cost-efficacy (benefit), he obtains the monetary value, the result of the relationship between the value of the resources invested and the value of the program made. Analysing the correlation advantage-unit and time unit, the physician observes that efficacy represents an economic manner of achieving a well-defined purpose.

The role of reason in using health re-

sources eases the difficult action of keeping records of the cost-advantage. For example, the role of personal hygiene in preventing infectious-contagious diseases, especially digestive ones, represents a logical, cultural, scientific and concise resource used rationally as an investment for health. Its absence generates higher costs in treating the inherent diseases. The quantitative decrease of the number of these diseases reduces the number of the ones who get ill, as well as the financial results (costs) due to the logical and positive use of the resource in question.

Of course, if the group it is addressed to is well analysed from multiple points of view: the members' cultural level, receptivity period, logistical and support component of theoretical investment etc.

The high costs in health represent one of the biggest problems. High quality medical services, the production of medical science with sophisticated means, the consumers' request for more and more sophisticated devices and solutions that are mainly logistical, contribute fully to this phenomenon. The cost-efficacy relationship is always relative, depending of the results of other programs or of a general rule.

In order to make an informed choice about if and how much to pay for a medicine, it requires evidence beyond the classical safety and efficacy data from the clinical trials. Compiling huge amounts of economic data, *electronic health records* (EHRs), genomic data, labor statistics etc., business players can better illustrate the value of their products. Two studies showed that half of American consumers are willing to pay more for personalized medical products but at the same time, are divided about paying for the tests that enable these treatments (cit. by 6).

The analysis of the relationship between

cost and utility is measured in precise results: the effect of treatment on the patient's life quality; years of life won depending of the quality of life; number of days without getting ill; number of lives saved, early identification of cancer etc. Considering this multitude of advantages at human level, we understand that the moral aspect of the problem comes first (6, 8).

But providing an etiological treatment, apart from its efficacy, it also induces the advantage of diminishing the financial value of those treatments that are difficult to define, reducing the labour incapacity period, the hospitalization period etc. When referring to maximum efficacy with the minimum of resources, by minimum we must not understand "insufficient" or "cheap", but a scientific, rational, attentive use of resources.

Returning to preventive investment, it proves to be less financially demanding than curative investment, although none of the components can be pointed out in the detriment of the other as they create a logical balance. Prevention interventions are not necessarily more effective than the ones that focus on treating. The prevention and curation % worldwide are almost equal. The idea is that the damage once created brings along a lot of human suffering and medical effort, including material and financial effort.

Considering its efficacy, any treatment program must be subjected first to a keen examination: to what extent it is feasible; if it prevents complications effectively; if it justifies itself from a humanitarian (reduces pain, deaths) and economic (reduces the number of patients) point of view; to what extent it influences life quality. The decrease of the number of deaths, of irretrievable biological losses that are impossible to compare and label depends of preventing

and counteracting the avoidable factors of post neonatal mortality with the aid of the managerial conception and intervention of health care services in correlation with social, economic, political and legal factors.

It results that any physician is invested with economic responsibilities even if he only writes down a recipe. Economic assessment is professionally necessary but also out of respect for the patient, as it represents a fundamental part of the decision making process. A good, useful, efficacious and effective use of resources depends of rational, scientific thinking that is the prime self-resource of total management. Resource management taken into custody must be credited with purpose, as an action, always compatible with similar activities.

HEALTH CARE AND INFORMATION TECHNOLOGY

While IT (*information technology*) has the potential to greatly improve the quality of health care, the evidence that IT improves important health related outcomes is limited. Some authors (9, 10) argument that while HIT (*health information technology*) has the potential to lead to a dramatic transformation in healthcare delivery, the empirical research showed limited results supporting HIT benefits. Their recommendations on a successful HIT implementation require a system that is intuitive to use and that requires little training for users; a system that can be modified and developed easily.

The authors also note that although there is an increasing number of a HIT solution designed to be used directly by the patient (for example, internet self-help sites), very little is known in terms of use and outcomes. Also, the main HIT implementation barriers are cost and overcoming misgivings from physicians due to per-

ceived adverse effects on their time.

The Integrated Health Information System of the Social Health Insurances goal is to ensure the computerized management of the economic and medical data necessary for an efficient Romanian health insurance system. Thus, it contributes to the development and improvement of the medical and pharmaceutical services.

System's coverage area: National Health Insurance House, all the County Health Insurance Houses, Physicians, Family Doctors, Medical assistants, Hospitals, Providers of ambulance services, Providers of homecare services, Out-patient recovery, Providers of medical devices, Providers of rehabilitation sanatoria services, Providers of pharmaceutical services.

IHIS (*Integrated Health Information System*) in Romania aims at improving the social health insurance system among others by collecting and managing the economic and medical information, ensuring the transparency regarding the control and management of the budgetary funds, creating and administering the National Registry of Insured Persons and the National Registry of Medical Services Suppliers. The main functionalities of IHIS include the management of the social health insurance funds, the management of the insurant, the management of the medical and pharmaceutical service providers, the evidence of taxpayers and control of the medical services.

However, the drive for more health data and integration comes also from the private sectors, as both consumers and physicians cheer the dramatic expansion of the personal medical kit, with wearable tech, smartphone-linked devices and mobile apps becoming increasingly valuable in health care delivery.

Personal medical kits could help diag-

nose illness, detect early signs of disease, and facilitate the recovery and rehabilitation closer to home. Clinicians seem more open to using these tools than consumers, according to HRI (*Health Research Institute*) survey findings. One-fifth of consumers said they would use a home urinalysis device. But nearly half of physicians said they would use data from such a device to prescribe medication or decide whether a patient should be seen. Nearly 90% of MDs said that patient devices and apps will be important to their practices in the next five years.

The app market – filled with 50,000 free and nearly-free products – is highly saturated. The Romanian market of smartphones had around 2.5 million units sold in 2014, and the tablets reached 700,000 units with at least as many potential users for health care apps.

PRIVACY AND SECURITY

All that may come to my knowledge in the exercise of my profession or outside of my profession or in daily commerce with men, which ought not to be spread abroad, I will keep secret and will never reveal. (The Hippocratic Oath, 4th Century BC) (11).

The number of websites and social networks providing health care related information and opportunities for patients to both share information and meet other patients is growing fast. For example, Yahoo health or WebMD, have around 20 million unique visits every month while in Romania, ro-medic.ro has over 1.5 million (1).

Several studies have showed the benefit of using Internet to share health-related information. Patients use the information to self-diagnose symptoms, understand the treatments and the medication devices (12) create or improve relationships (13), and deal with symptoms.

As the volume of personal information captured and digitized continues to grow at exponential rates, the concept of privacy has also been put on the top of the list of concerns for the public opinion. Recent research highlighted the significance of privacy concerns over the internet sharing of health or intimate data or how individuals deal with threats to their electronic privacy. Another author believes that privacy concerns could be the greatest threat to successful rollouts of EHRs.

At the same time with the increased shared and “in the cloud” patient and health data, the proliferation of medical devices in patients’ homes, and apps on their phones points to an even more pressing need for strong information security systems.

In summer of 2014 alone, over five million US patients had their personal data compromised in health system privacy breaches. As for the mobile eco-system, the US Department of Homeland Security issued a warning to police officers, firefighters, emergency medical services and security personnel about the security issues in Android. The report says Android accounts for 79 percent of mobile malware, while Apple’s iOS accounts for 0.7 percent. According to analysis company Stat Counter, Google’s Android platform is the clear leader in Romania, reaching 62.73% market share in September 2014.

Still, the consumers want one-click access to their data and the challenge is to find the right balance between privacy, security and ease of use. Over 65% of respondents in a research said data security was more important to them than convenient access to imaging and test results, doctor’s notes, diagnoses and prescriptions. For fitness data, the reverse was true. However, the consumers could agree to share health data if they would see social or in-

creased care value in doing so.

Future cyber security safeguards will have to focus on what consumers look for – health data that is private, secure and one click away.

HEALTH CARE ECONOMICS – FUTURE DIRECTIONS AND CONDITION OF SUSTAINABLE HEALTH

The complete confidence with which a physician establishes the correct diagnosis determines the number of hospitalization days, the cost of the treatment, the number of recovering days, briefly, the efficacy of the medical act which is an important indicator for economic sciences.

Consequently, in spite of the fact that the economic factor is not the one guiding health, its decisive role cannot be ignored. From this point of view, Romania is facing severe problems. This situation will become even worse considering that the health care system confronts itself with three different pressure sources: the justified expectations of the population, which asks for a fundamental right of human beings; the progress of medical technology represented by equipment’s, therapies and drugs, research studies in this field, which require important investments; population ageing (the costs with an older patient are 5-10 times higher than with a younger patient) (12, 14).

Seen from the economist’s point of view, health, although it does not have a material meaning, is absolutely necessary for social and economic development, the economic activity being, by excellence, a support for protecting and conducting health care activities.

Health must be included in the political, economic, social, environmental and institutional context, the strategic frame being oriented toward accomplishing actions for

improving the state of health and assessing the impact of health care.

The economic principle applies to all the dimensions of the disease (apparition of pathogenic agents; their adjustment to the organism's defence mechanisms, to the artificial antimicrobial means; recombination of viruses; multi-causality and multi-diversity) and of health (preventing complications, premature death, disabilities, infirmities, diseases) (12).

Promoting health endows people with the best ways of ensuring and improving their own state of health. Health is not the purpose of life but one of its resources and it must be promoted through social responsibility, more investments and partnerships and the development of an appropriate infrastructure.

The promotion of health includes flexible models, adjusted to the present and future demographic situations, all of this in the general frame of the principles applicable to all people and all parameters that condition health care.

The health price and the disease cost are established based on an analysis that has at its base the medical and economic principles of efficacy and efficiency. The health price requires a longer period of time, even decades to be accomplished. It is cheaper if moral investments are made, first of all, common sense in promoting health for the individual's physical, mental and social state of good. The disease cost refers to the disadvantages it brings at human level (13).

The priority for health economics in the process of providing health care services resides in the economy of human lives. Promoting health is an economic segment of the financial – material investment, and on the other hand, health structure's relies on efficacy and efficiency.

Any attempt of restructuration of the

health care system in Romania must begin from the analysis of the way in which the ones financially supporting the health system think and react; the health subsystem, as part of the global social system, relies on market economy, on the contributor's financial effort. The quality and the efficacy of the ones coordinating the professional destinies of the health care system must provide a convincing answer, based on the criteria and the methodology of an economic analysis at the level of health care services and individual practice. It is very important to be aware of the utility, the purpose of the activity, the patient's satisfaction and the contribution to the global health of the two essential factors: ethics and economic under-layer.

In Romania, decentralization and the reform of the financing system were made without a serious analysis of the consequences on the population in terms of territorial inequity and of the availability of the infrastructures and implicitly of the services. Pros and cons (*pro et contra*) for decentralization of the health care system:

➤ *Pros* (advantages):

- Subsystems can be organized considering the local values, available infrastructure, diseases, preferences and services;
- Increase the income in the community (local) level and the efficiency in resources allocation
- Decentralized units can be used as "laboratories" for national policies.

➤ *Cons* (disadvantages):

- Possible additional costs;
- System fragmentation;
- Decisional centralism vs. Administrative decentralization;
- Misappropriation of national programs or institutions redundancy.

NSPHHSMPD (SNSPMS - *National School of Public Health and Management Bucharest*) contributes to improving the performance of health care services and improving the health of the population in Romania by consulting services and technical assistance to decision makers and providers of healthcare services, offered in order to optimize health policies and health services and developing health promotion programs with the purpose of responsibility and increasing health education among population.

Main actions include providing scientific evidence to support health decisions and policies; evaluation, application and dissemination of research results to improve health practices; conducting studies and operational research in order to increase health system performance; educating and informing the population on attitudes and behaviors related to health, healthy lifestyle and public awareness about the importance of individual involvement in the maintenance and/or improvement of the health status.

Short and medium-term steps for health care system reform in Romania should focus, among others on:

a. Streamlining hospital services, im-

plementation of regional hospitals networks, and reducing redundant capacity for inpatient services;

b. Enhancing primary health care services at the community level;

c. Implementing specialized secondary ambulatory care;

d. Increasing sector governance and stewardship to: strengthen the implementation of HTAs improve the management control mechanisms; implement health system continuous quality improvement and health system performance assessment; adjust the DRG (*diagnosis-related groups*) payment mechanism; streamline the National Health Programs; create the conditions for increasing the participation of private funds and private services; develop a Human Resource Strategy; strengthen the communications strategy

Examples of Public Health Schools with HTA dedicated departments or programs: University of Alberta, UMIT (University for Health Sciences, Medical Informatics and Technology, Tirol, Austria), Harvard School of Public Health, University of Birmingham, University of Adelaide, Aarhus University, and National University of Singapore.

REFERENCES

1. Bonciu C. *Instrumente manageriale psihosociologice*. All Beck Publishing House, 2000.
2. Burduș E, Căprărescu G. *Fundamentele managementului organizației*. Economica Publishing House, Bucharest, 1999, 181–209.
3. Getzen TE. Macroeconomic Dynamics of Health: Lags and Variability in Mortality, Employment, and Spending. *Encyclopedia of Health Economics*, Elsevier 2014; 165-176.
4. Haines A. Health benefits of a low carbon economy. *Public Health* 2012; 126, Suppl. 1, S33-S39.
5. Ionescu G, Cazan E, Negrușă AL. *Management organizational*. Tribuna Economica Publishing House, Bucharest, 2001.
6. Marcu M, Minca DG. Sănătate publică și management sanitar – note de curs pentru învățământul postuniversitar. “Carol Davila” Publishing House, Bucharest, 2002.
7. Garrido MV, Kristensen FB, Nielsen CP, Busse R. Health Technology Assessment and Health Policy-Making in Europe Current status, challenges and potential. World Health Organization 2008, on

- behalf of the European Observatory on Health Systems and Policies. Available online at: http://www.euro.who.int/__data/assets/pdf_file/0003/90426/E91922.pdf [Accessed on: 29.01.2016]
8. Rugină V. *Curs de Medicină Socială*. Iasi Publishing House, 1986.
 9. Ortiz E, Clancy MC. Use of information technology to improve the quality of health care in the United States. *Health Serv Res*. 2003; 38(2): xi–xxii.
 10. Shekelle PG, Goldzweig CL. Costs and benefits of health information technology: an updated systematic review. The Health Foundation UK, 2009. Available online at: <http://www.health.org.uk/publication/costs-and-benefits-health-technology-information> [Accessed on: 28.01.2016].
 11. Jacob JM. *Doctors and Rules: A Sociology of Professional Values*. London: Routledge, 1988 (Selection available online at: https://books.google.ro/books/about/Doctors_and_Rules.html?id=ZD1NIErWURgC&redir_esc=y) [Accessed on: 27.01.2016].
 12. Vulcu L. *Economia sănătății pentru medici*. "Lucian Blaga" Publishing House, Sibiu, 2004.
 13. Vulcu L. *Management sanitar*. "Lucian Blaga" Publishing House, Sibiu, 2004.
 14. Ruhm CJ. Macroeconomy and Health. In: *Encyclopedia of Health Economics*, Elsevier, 2014, 181-186.

NEWS

CLOSER AND CLOSER TO THE IDEAL BIOMARKER IN INFLAMMATORY BOWEL DISEASES

The role of the intestinal microbiota in the pathogenesis of inflammatory bowel disease (IBD) is more and more taken into consideration. Biomarkers obtained from alterations in the gut microbiome may be an alternative marker of disease presence and severity. A recent study showed that Trimethylamine-N-oxide (TMAO) production is dependent on the metabolism of GI tract microbiota. A case-control study carried out for 106 IBD patients and 373 non-IBD controls from southwestern Ontario found plasma TMAO levels to be significantly lower in patients with IBD. With further research, plasma TMAO levels may become a relevant, cheap, noninvasive means of diagnosing IBD (Wilson A, Teft WA, Morse BL, Choi YH, Woolsey S, DeGorter MK, Hegele RA, Tirona RG, Kim RB. Trimethylamine-N-oxide: A Novel Biomarker for the Identification of Inflammatory Bowel Disease. *Dig Dis Sci*. 2015 Dec;60(12):3620-30. doi: 10.1007/s10620-015-3797-3)

Iolanda Popa

INTRAUTERINE GROWTH RESTRICTION – PREDICTIVE SERUM MARKERS

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INTRAUTERINE GROWTH RESTRICTION – PREDICTIVE SERUM MARKERS (Abstract) **Aim:** To determine during the first trimester of pregnancy some serum and ultrasound markers that could improve perinatal morbidity and mortality in women with intrauterine growth restriction (IUGR). **Material and methods:** Prospective study of pregnant women gestational age 11 weeks and 0 days - 13 weeks and 6 days, consisting in the determination of pregnancy associated plasma protein (PAPP-A), mean platelet volume (MPV), and ultrasound appearance of placenta. **Discussion and Conclusions:** This study suggests that screening by detailed history and PAPP-A and MPV determination during the first trimester of pregnancy in women at risk for IUGR makes possible the prophylactic treatment and monitoring of pregnancy according to a given protocol and thus neonatal morbidity and mortality to be reduced. **Keywords:** INTRAUTERINE GROWTH RESTRICTION, PREGNANCY-ASSOCIATED PLASMA PROTEIN, MEAN PLATELET VOLUME.

Detection of IUGR risk in pregnant women in the first trimester is an important goal of research in modern obstetrics. It is known that fetuses diagnosed with IUGR are at high risk of perinatal morbidity and mortality by the complications that may occur (1,2,3).

In this context the early determination of the predictive markers for the risk of IUGR is essential. First trimester screening consists in the monitoring of pregnant women at risk according to a secondary prevention protocol and monitoring specific to each associated disorder so that the following two situations that affect fetal prognosis to be avoided:

✓ premature, unnecessary extraction of

a healthy fetus

✓ delayed extraction due to fetal distress undetected on time and which can result in intrauterine fetal death

Evaluation of the predisposing/causative factors for IUGR is the first step and consists in history taking, thorough physical examination, ultrasound, and specific laboratory tests, all aimed at ultimately determining the presence or absence of the risk for IUGR (4-16)

PAPP-A is a protein secreted by the syncytiotrophoblast cells and maternal decidua, thus low PAPP-A levels are associated with abnormal placentation and fetal growth (17-22).

MPV – Increased MPV reflects en-

hanced platelet activation, which may be caused by impaired uteroplacental circulation, and inadequate trophoblastic invasion, associated with an implicit risk of preeclampsia (PE) and IUGR (23-25)

MATERIAL AND METHODS

This prospective study was conducted in 70 women 11 weeks and 0 days -13 weeks and 6 days gestational age monitoring their pregnancy at the Neamt County Emergency Hospital between August 1, 2014 and September 1, 2015.

Throughout their pregnancy the women included in the study received prenatal care:

- ultrasound examinations (at 12 -13 weeks - fetal morphology, fetal development and placental appearance, at 22-24 weeks – fetal morphology, uterine artery Doppler study, at 32-34 weeks – fetal well-being study), repeated depending on fetal status and pregnancy-related disease;
- laboratory tests according to the protocol: complete blood count, MPV, blood glucose level, urea, creatinine, uric acid, transaminases, anti-toxoplasma antibodies (Ab), rubella Ab, anti-herpes Ab, cytomegalovirus (CMV), urinalysis, repeated depending on pregnancy-related disease.
- double test as part of biochemical screening (PAPP-A and beta-human chorionic gonadotropin (B-HCG) hormone.

The study aimed to determine the PAPP-A, MPV and ultrasound placental changes, as potential predictors of the risk for IUGR.

Each participant signed and informed consent form approved by the ethics committee.

Inclusion criteria for participation in the study were:

- 11 weeks and 0 days -13 weeks and 6 days pregnant women at a craniocaudal length (LCC): 44,5 mm – 84.5 mm)

- Normal fetal morphology

Exclusion criteria:

- Double tests with high risk for trisomy 13, 18, 21
- Fetal abnormalities diagnosed with ultrasound in the first trimester
- Genetic abnormalities confirmed by chromosomal analysis of cells obtained by chorionic villus biopsy.
- Pregnancy achieved through in vitro fertilization (IVF)
- Twin pregnancy.

PAPP-A was determined as part of first-trimester biochemical screening for aneuploidy at a LCC of 44,5 mm – 84,5 mm. Samples were processed with Brahms Kryptor immunoassay analyzer. The results were expressed as multiples of median (MoM).

The data were imported into, processed and analyzed using the SPSS version 18.0, and were statistically significant at 95% confidence level. Chi-square test was used to determine statistical significance. Frequencies and mean values for the quantitative and qualitative determinations were calculated and Pearson and Fearman tests were used to find the correlations between several variables. The difference was considered statistically significant at $p < 0.05$.

RESULTS

This study was conducted in a group of 70 pregnant women monitored at the Neamt County Hospital in the interval August 1, 2014 - September 1, 2015. Pregnancies were dated by LCC at first ultrasound, LCC ranging from 44.5mm to 84.5mm.

Physical examination and history identified the following pregnancy-associated disorders and predisposing factors of IUGR: thrombophilia in 8 cases, uterine fibroma 1 case, pre-existing thrombocytopenia 1 case, iron deficiency anemia 7 cases, preexisting hypertension 11 cases, type 1 diabetes 7

cases, and smoking in 11 cases.

The 70 pregnant women included in this study gave birth to 41 small-for-gestational age pregnancy (SGA) and 29 normal weight babies (fig. 1). Of the 41 cases with IUGR, 30 had a first trimester PAPP-A <0.5 MoM, confirming the statistically significant correlation between maternal PAPP-A level and birth weight ($p=0.016$).

At the baseline ultrasound performed at 12-13 weeks gestation the ultrasound appearance of placenta was also assessed

(presence of decidual hematoma, areas of angiomatous transformation, detachment of placenta), which suggests impaired first wave of trophoblast invasion and thus a possible long-term placental insufficiency and implicitly risk of IUGR. In 25 cases such changes were identified.

Thirty of the infants had low birth weight and first trimester MPV levels > 10 fl, (fig. 2). A statistically significant correlation was found between birth weight and first trimester MPV level ($p = 0.001$).

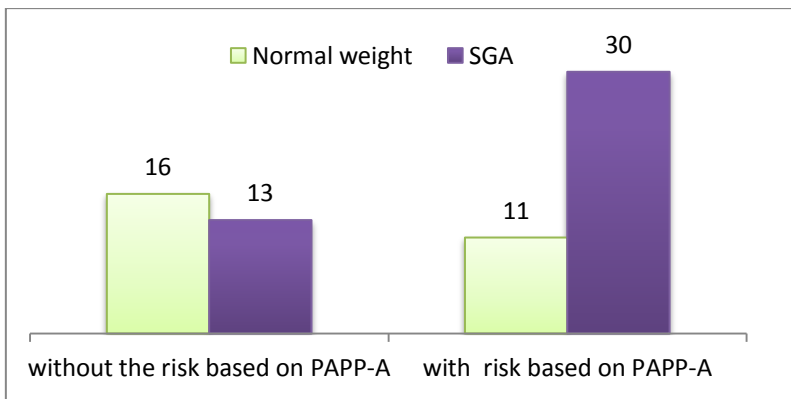


Fig. 1. Distribution of cases by birth weight and PAPP-A

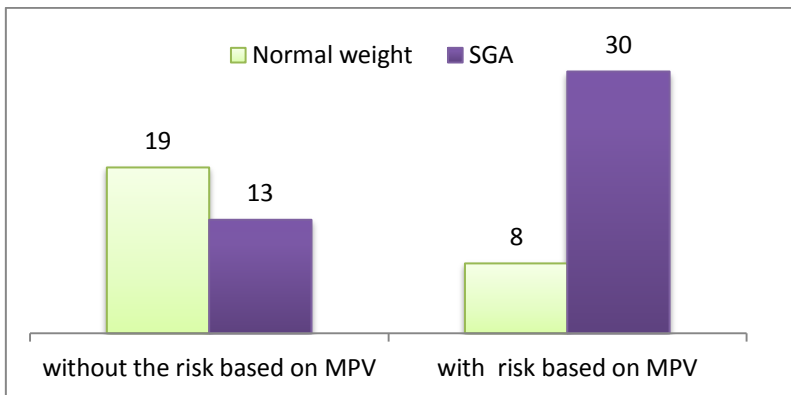


Fig. 2. Distribution of cases by birth weight and MPV

DISCUSSIONS

IUGR is a current problem and a research topic in modern obstetrics. In prac-

tice, IUGR has a statistical definition and is a fetal weight or fetal biometry below the 10th percentile or 2 SD below the mean for

gestational age (3, 4). According to the data in the literature, elevated PAPP-A levels are not correlated with increased risk of obstetric pathology, but it was found that a PAPP-A level below the 5th percentile is associated with increased risk of IUGR (17-22).

A study by M. Kwik in 894 women in the first trimester of pregnancy showed that serum PAPP-A levels <0.5 MoM have a sensitivity of 33% and a specificity of 89% in detecting fetuses with a weight below the 10th percentile. Low PAPP-A levels indirectly indicate abnormal placentation, that is a placental insufficiency which has effects on fetal growth (19).

In our study 30 of the pregnant women with IUGR had PAPP-A levels <0.5 MoM, suggesting a statistically significant correlation between IUGR and PAPP-A ($p = 0.016$).

Increased MPV reflects platelet activation caused by uteroplacental circulatory insufficiency, pathophysiological mechanism with repercussions on fetal growth. In this study it was found that 30 newborns had low birth weight and a first trimester MPV level > 10 fl, $p = 0.001$, data consistent with the literature (23-25).

A study by M. Kanakt-Pektas shows that MPV levels > 10 fl can predict IUGR with a sensitivity of 82.4% and specificity of 60% (24).

In our study, 41 pregnant women had PAPP-P levels <0.5 MoM and in 38 cases MPV level was > 10 fl, which, according to the above mentioned literature data, can be used as markers for the early prediction of

IUGR.

The ultrasound appearance of impaired first wave of trophoblast invasion is described as intra-and/or periplacental pathological areas which can be considered a possible cause of placental insufficiency, thus a risk of IUGR.

CONCLUSIONS

This study shows that the early detection of serum markers PAPP-A, MPV and placental changes detected at ultrasound and the detailed medical history obtained during the first trimester of pregnancy can be used as screening markers for the detection of cases at risk for IUGR. Identifying pregnancies at risk for IUGR during the first trimester requires the use of secondary preventive measures and monitoring of these cases in order to reduce neonatal morbidity and mortality.

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REFERENCES

1. Mandruzzato G, Antsaklis A, Botet F, Chervenak FA, Figueras F, Grunebaum A, et al. Intrauterine restriction(IUGR). *J Perinat Med* 2008; 36: 277-81.
2. American College of Obstetricians and Gynecologists. ACOG Practice bulletin no. 134: Fetal growth restriction. *Obstet Gynecol* 2013; 121: 1122-1133.

3. Anghelache Lupascu I. *Obstetric ultrasound*. Iasi: European Institute, 2003, 85-94.
4. Tsatsaris V. – Le retard de croissance intra-utérine, Aspects cliniques et fondamentaux. Paris: Elsevier Masson S.A.S, 2012.
5. Albu AR, Anca AF, Horhoianu VV, et al. Predictive factors for intrauterine growth restriction. *J Med Life* 2014; 15: 165-171.
6. Salomon L.J. , Malan V. , Bilan étiologique du retard de croissance intra-utérin (RCIU). *J Gynecol Obstet Biol Reprod* 2013; 42: 929-940.
7. Gaudineau A.. Prévalence, facteurs de risque et morbi-mortalité materno-fœtale des troubles de la croissance fœtale. *J Gynecol Obstet Biol Reprod* 2013; 42: 895-910.
8. Nizard J. . Prévention du RCIU vasculaire. *J Gynecol Obstet Biol Reprod* 2013;42: 1008-1017.
9. Mafina-Mienandi M.C., Ganga-Zandzou P.-S., Makoumbou P., Malonga H., Ekoundzola J.-R, Mayanda H.-F.. Facteurs de risque de retard de croissance intra-utérin au Congo. *J Gynecol Obstet Biol Reprod* 2002; 31: 500-505.
10. Davitian C. , Uzan M. , Tigaizin A. , Ducarme G. , Dauphin H. , Poncelet C. . Consommation maternelle de cannabis et retard de croissance intra-utérin. *UUJMS* 2006; 34: 632–637.
11. Collet M., Beillard C.. Conséquences du tabagisme sur le développement fœtal et le risque de retard de croissance intra-utérin ou de mort fœtale in utero. *J Gynecol Obstet Biol Reprod* 2005; 34: 135-145.
12. Heilbronner C., Quelles sont les conséquences à court, moyen et long terme du tabagisme pendant la grossesse ? *J Gynecol Obstet Biol Reprod* 2005; 34: 390-446.
13. Mezane S., Achenani M., Benabdjalil Y., Ziyadi M., Hafidi R., Hakimi I., A. Grossesse sur uterus bicorne menee a terme apropos d'un cas avec revue de la litterature. *GJ Med res e Gynecol Obstet* 2014; 14: 35-38.
14. Quibel T., Bultez T., Nizard J., D., Huchon C., Rozenberg P.. Morts foetales in utero. *J Gynecol Obstet Biol Reprod* 2014; 43: 883-907.
15. Vanlieferinghen S., Bernard J.-P., Salomon L.J., Chalouhi G.E., Russell N.E., Ville Y.. Retard de croissance intra-utérin du deuxième trimestre : malformations associées et syndromes sous-jacents, *Gynecol Obstet Fertil* 2014; 42: 567-571.
16. Cordier A.-G., Nedellec S., Benachi A., Frydman R., Picone O., Quels arguments pour déterminer l'origine infectieuse d'un retard de croissance intra-utérin ? *J Gynecol Obstet Biol Reprod* 2011; 40: 109-115.
17. Geyl C., Subtil D., Vaast P., Coulon C., Clouqueur E., Deruelle P., et al. Interprétation des valeurs atypiques des marqueurs sériques. *J Gynecol Obstet Biol Reprod* 2014; 43: 5-11.
18. Carbone JF, Tuuli MG, Bradshaw R, Liebsch J. Efficiency of first-trimester growth restriction and low pregnancy associated plasma protein-A in predicting small for gestational age at delivery. *Prenat Diagn* 2012; 32: 724–729.
19. Kwik M., Morri J., Association between first trimester maternal serum pregnancy associated plasma protein-A and adverse pregnancy outcome. *Aust N Z J Obstet Gynaecol* 2003; 43: 438–442.
20. Conde-Agudelo A, Papageorgiou A.T, Kennedy S H, Villar J. Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis. *BJOG* 2013; 120: 681-694.
21. Goetzinger KR, Singla A, Gerkowicz S, Dicke JM, Gray DL, Odibo AO. The efficiency of first-trimester serum analyses and maternal characteristics in predicting fetal growth disorders. *Am J Obstet Gynecol* 2009; 201: 412-416.
22. Poon LC, Maiz N, Valencia C, Plasencia W, Nicolaides KH. First trimester maternal serum pregnancy associated plasma protein-A and preeclampsia. *Ultrasound Obstet. Gynecol* 2009; 33: 23-33.
23. Han L, Liu X, Li H, Zou J, Yang Z, Han J. Coagulation Parameters and Platelet Indices:Changes in Normal and Preeclamptic Pregnancies and Predictive Values for Preeclampsia. *PLOS ONE* 2014; 9: 1-14
24. Kanat-Pektas M, Yesildager U, Tuncer N, Arioz DT, Nadiirgil-Koken G. Could mean platelet volume in late first trimester of pregnancy predict intrauterine growth restriction and pre-eclampsia? *J. Obstet. Gynaecol. Res* 2014; 40: 1840–1845.
25. Missfelder-Lobos H, Teran E., Lees C., Albaiges G., Nicolaides K. H. Platelet changes and subsequent development of pre-eclampsia and fetal growth restriction in women with abnormal uterine artery Doppler screening. *Ultrasound Obstet Gynecol* 2002; 19: 443–448.

AXIAL MODIFICATIONS OF PERMANENT LOWER MOLARS AFTER PREMATURE LOSSES OF TEMPORARY MOLARS

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AXIAL MODIFICATIONS OF PERMANENT LOWER MOLARS AFTER PREMATURE LOSSES OF TEMPORARY MOLARS (Abstract): **Aim:** The aim of our study was to determine the impact of premature loss of temporary lower molars upon the longitudinal axis of the first and second permanent molars. **Material and Methods:** The study groups included 61 patients, 6 -9 year olds with premature loss of primary molars and a control group of 24 patients with intact temporary teeth. We evaluated the angle between longitudinal axis of first and second lower permanent molars and occlusal plane. **Results.** It was observed that premature loss of lower second deciduous molar modifies more the vertical axis of first and second permanent molars than the premature loss of first lower primary molar. Reducing space occurs mainly through mesial inclination of molars that separates the edentulous breach. Temporary loss of both lower first molars on the same quadrant causes an accelerated eruption of both premolars increasing the prevalence of eruption sequence: “4-5-3-7”. **Conclusions:** The preservation of the occlusal morpho-functional complex using space maintainers mainly when the premature loss of the second primary molars occurs is the best interceptive treatment option. **Keywords:** PREMATURE LOSS, AXIAL MODIFICATION, PERMANENT LOWER MOLARS, DENTAL CARIES, TEMPORARY TEETH.

The first one who debated upon the subject of premature loss of temporary teeth were *Hutchinson* and *Davenport* as early as the 1880s (1,2). The concept was defined as the exfoliation of deciduous teeth on the arch with more than 12 months prior to the normal eruption period of permanent teeth, exceeding the limits of normal variability of temporary teeth exfoliation sequences (3).

The most frequent causes that trigger the premature loss of temporary teeth are: untreated complicated dental cavities, traumatism and premature root resorption.

The premature loss of temporary teeth, as a consequence of untreated dental cavity of temporary teeth may lead to serious tridimensional dental, dento-dental, dento-alveolar, occlusal and variably skeletal lesions. Thus, we control the migration of permanent teeth during intra-maxillary or oral eruption, the consequence being dento-alveolar incongruence. There may also appear super-eruptions of antagonist teeth, arch narrowing, reverse toothing, premature contacts and occlusal interferences (4).

Studies have shown that the premature

loss of temporary molars I mainly determines the distal migration of temporary canines. In this case, mesial displacement of permanent molars or their version cannot occur (5). Greater effects appear at the moment of the premature loss of second temporary molars, as they serve as a guide for the eruption of the permanent molar (6). The premature loss of second temporary molars – mainly mandibular - determines a shrinking of the arch length due to mesial inclination of permanent molars, compromising the „lee-way space” by affecting the posterior support area (7). The radiologic exam is mandatory for these patients. Although the cone beam computed tomography (CBCT) has progressively become a radiological method or reference in the oro-maxillo-facial sphere, the orthopantomography still represents a routine exam for children under 12 and not only. It thus represents a simple and economical way that uses a low radiation dose in comparison with the CBCT and allows a rapid quantitative and qualitative analysis both of the bone and the dental structures, allowing at the same time the quantification of the inclination degree of permanent molars in the context of the premature loss of temporary molars.

The main purpose of this study is to determine the impact of premature loss of mandibular temporary molars on the longitudinal axis of the permanent molars. A secondary objective is to verify whether there appear important modifications in the eruption sequence of the successors and the way the axis of the permanent molars is influenced.

MATERIAL AND METHODS

The study included 85 orthopantomographies of subjects aged 6 to 9 who reported to the Clinic of Pediatric Dentistry, Iasi.

Out of these, 61 (71.8%) patients showed a premature loss of a lower temporary molar (study group) and 24 (28.2%) had intact dentition (control group). The study group was re-divided in all possible subgroups depending on the premature unilateral or bilateral loss of temporary molars. The precondition for the selection of subjects was that the incisors and permanent molars were intact. All patients received the required treatment and their written, informed and motivated approval was obtained.

All orthopantomographies were made at a single radiologic center by the same experienced technician in ideal conditions, using the radiologic device *PaX-Uni3D(Vatech)*®. At the moment of the orthopantomography the patients needed to have relaxed lips, a correct position of the head in relation to the chin support and the Frankfort plane needed to be parallel with the horizontal plane. Low quality, improper films or those with artifacts were excluded from the study from the beginning and the valid ones were saved digitally. Measurements were made by a single examiner using the *Easy Dent 4 Viewer*® software.

The orthopantomographies were processed by tracing the following structures (fig. 1):

- the occlusion plane* – tangent line to vestibular cuspids of temporary molars/premolars and disto-vestibular cusp of lower right/left permanent molar;

- the axis of primary permanent molar (M1)* – the line passing through the center of the crown and the center of radicular bifurcation;

- the eruption axis of the second permanent molar (M2)* – the line passing through the middle of the crown and the center of the starting point of the radicular bifurcation.

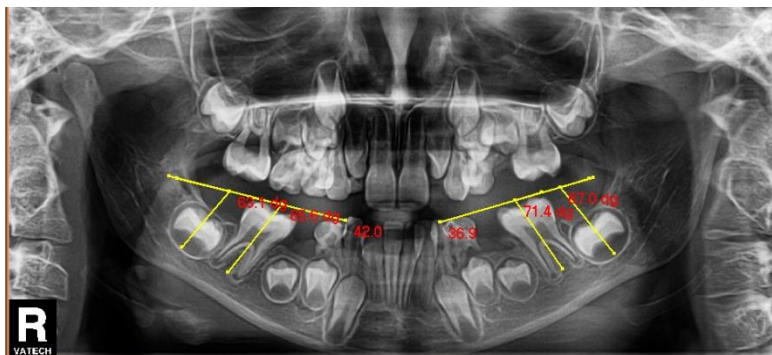


Fig. 1. Orthopantomographic aspect of premature loss of temporary molars

We thus evaluated the angle formed between the longitudinal axis of the first and second permanent molar and the occlusal plane in both lower quadrants. The software used for the data analysis was Statistical Package for the Social Sciences (version 20.0; SPSS, Chicago, III). We calculated the frequency and descriptive parameters (mean value, standard error, standard deviation, the minimum and maximum values) for each group. We used the *t-student test* to compare each group with the control group. The *t-pairs test* was used to compare the first permanent lower molars (M1) axes with second permanent lower molar (M2) axes of the two quadrants. The error limits used in the statistic interpretation are of 5% and the reliability range was of 95% .

In order to identify the possible errors during measurements we selected 25 radiographies. Measurements were repeated for the same radiographies and the *t-paired test* was used for the first and second measurement. No statistically significant difference was identified between the two measurements ($p>0.05$).

RESULTS

Out of the total 85 orthopantomographies analyzed, 45 (53%) patients were males

(with an age average of 7.29) and 40 (47%) patients were females (average age 7.38). As far as the age distribution of subjects is concerned, there is a prevalence of patients aged 7 to 8 with an average age of 7.33.

In our study a total of 75 lower temporary molars were lost prematurely due to untreated dental cavities. The cumulated frequency of first temporary molar (m1t) and second temporary molar (m2t) losses shows a value of m1t close to m2t (37 (49.5%) for m1t and 38 (50.5%) of m2t).

From the point of view of angulation, the mesial-version mean for the control group of lower permanent M1 is of 80.38° (variation 69° - 87°) and for lower permanent M2 of 69.74° (variation 50° - 84°).

By analyzing the influence of *unilateral premature loss of lower temporary molars* on the inclination of permanent molar we may notice that the loss of lower m1t has very little influence on the axis of lower permanent molars (mean inclination of 80.078° , with a variation between 73° and 85.4° for M1 respectively a mean inclination of 65.47° with a variation between 50° and 81.50° for M2). The loss of lower m2t determines an accentuated mesial-version of permanent molars (angular mean value of 77.29° with a variation between 65.40° and 85.50° for permanent M1 and

respectively a mean value of 64.24° with a variation between 30° and 83° for permanent M2). In case of loss both of m1t and of m2t, the angle between the longitudinal

axis and the occlusal plane will increase close to the values of the control group up to mean angular values of 79.275° for M1 and of 68.75° for M2 (fig. 2).

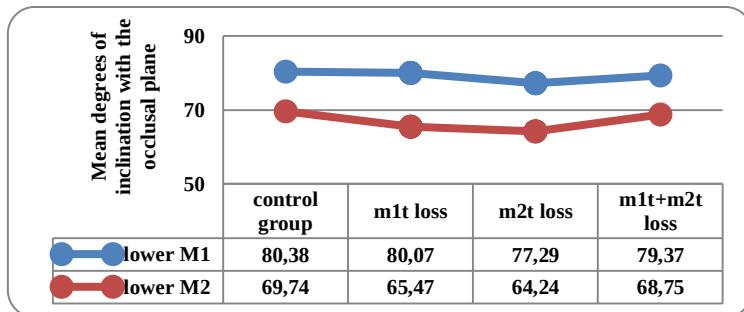


Fig. 2. Influence of unilateral premature loss of lower temporary molars on the inclination of permanent molars

The influence of *bilateral premature loss of lower temporary molars* on the inclination of permanent molars determines a mesial version of permanent molars in cases of bilateral loss of m2t (mean angular values being of 72.25° for M1 and of

60.91° for M2). Bilateral loss of both m1t and m2t influences positively the axis of permanent molars, determining in these cases a slight overhang towards the distal (mean angular values being of 81.15° for M1 and of 74.75° for M2) (fig.3).

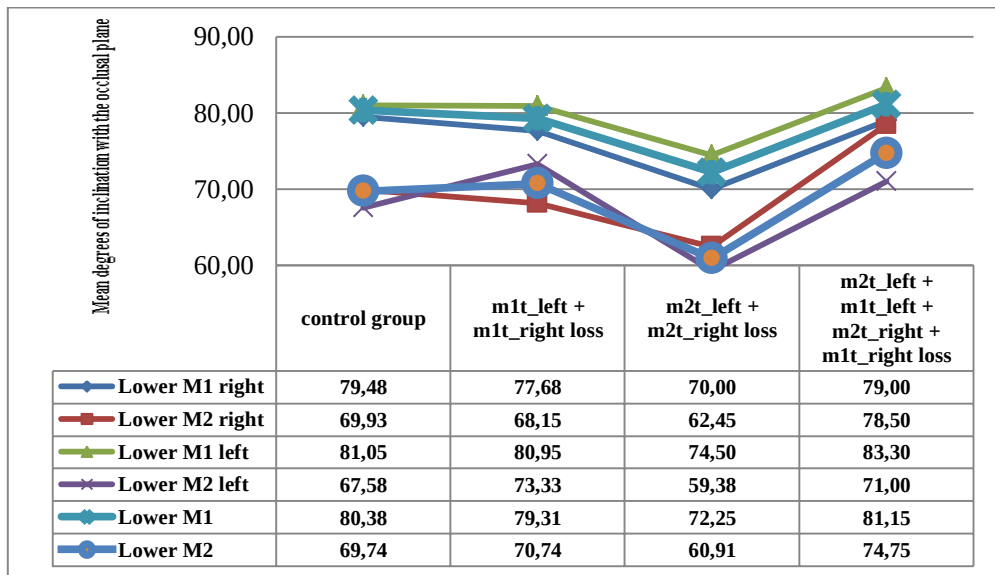


Fig. 3. Influence of bilateral premature loss of lower temporary molars on the inclination of permanent molars

Using the *t*-test we compared the axes of permanent molars, in the case of reunited global groups with premature loss of lower m1t, of lower m2t and of lower m1t+m2t, namely with the axes of perma-

nent molars on the same side of the control group. The only statistically relevant situation is the loss of lower m2t which influences both permanent M1 axis and the permanent M2 axis (tab. I).

TABLE I
Results of the comparison between the group with premature loss of m2t and the control group

	Groups compared	N	Mean	Std. Deviation	Std. Error Mean	p –value
Lower M1	m2t global	36	76.189	6.5877	1.098	0.021*
	control	24	79.913	4.8021	0.9802	
Lower M2	m2t global	36	65.431	11.789	1.9648	0.04*
	control	24	71.354	8.8143	1.7992	
Lower M1	m1t global	33	81.424	4.7743	0.8311	0.643
	control	24	80.863	4.069	0.8306	
Lower M2	m1t global	33	70.933	9.0698	1.5788	0.247
	control	24	68.129	8.7279	1.7816	
Lower M1	m1t+m2t global	22	79.682	4.8291	1.0296	0.373
	control	24	80.863	4.069	0.8306	
Lower M2	m1t+m2t global	22	71.155	12.1297	2.5861	0.334
	control	24	68.129	8.7279	1.7816	
*Significant difference < 5.0%						

The correlative analysis between M1 axis and M2 axis in the two quadrants was made by comparison to the control group, using the *t* test for pairs (*t*-pairs). In both cases there is a moderate correlation, but statistically significant between the M1 and M2 axes (axis right M1 & axis right M2, $r=0.321$, $p=0.01$; axis left M1 & axis left M2, $r=0.510$, $p=0.000$).

DISCUSSIONS

The premature loss of temporary molars has as a main consequence the reduction of the space requires for the eruption of premolars and for the correction of small frontal dento-alveolar incongruence which is normally accomplished due to the leeway-space (8). The greatest amount of

space lost is due to the inclination of permanent molars towards the mesial. This inclination depends mainly on the dental age at the moment of the extraction but also on the sequence/periodicity of eruption (9-11).

Based on the analysis using the software we could observe with higher accuracy the angle formed by the longitudinal axis of the permanent molars with the occlusal plane and we could thus estimate a mean value, a minimum, maximum value and a standard deviation for the control group. A similar study which was conducted using an older technique, namely by tracing the teeth images and structures on tracing paper using a black pencil and the measurement of angles with the protractor,

considers that, for instance, the correct position of lower molar 1 is at an angle that ranges from $+80^{\circ}$ - $+100^{\circ}$ (12). The values we obtained for the lower M1 are similar with those in the previous study: the mean value being of $+80.38^{\circ}$ with a variation between $+69^{\circ}$ - $+87^{\circ}$. These aspects are relevant from the point of view of the interrelation between the moment of premature loss of m2t and the moment of initiation of eruption of the first permanent molar. The context of premature loss of second temporary molar occurred after the initiation of clinical eruption of the first permanent molar.

Many longitudinal studies for a period from 2 to 8 months on children aged 6 to 9 with *premature loss of the first lower temporary molar* concluded that the shrinking of the mesial space from the second molar is due mainly to the distal displacement of the temporary canine and less to the migration or inclination of permanent molars (13-16). These results are also confirmed by us, since the axes of the lower permanent molars were very little influenced after the loss of first temporary molars, being comparative with the values obtained for the control group. Thus, the mean angular value for lower M1 at the control group was of 80.38° while in case of m1t loss the mean value was of 80.07° , and for lower M2 at the control group the mean angular value was of 69.74° as compared to the mean angular value in case of m1t loss of 65.45° .

Many studies (9-11,16) showed that the *premature loss of second temporary molars* determines a greater loss of leeway-space than in the case of first temporary molar, due both to the bigger difference in the mesio-distal diameter and to the fact that the first and second permanent molar lose the contact point and follows the trend of

all teeth to lean mesially. This is also proved by our study by the much lower angular mean values for permanent mandibular molars in case of premature loss of the second mandibular temporary molar as compared to the mean angular values from the control group.

As a matter of fact, the only instance in our study which is statistically significant is the loss of lower m2t which influences the axis of both lower permanent molars, a logical consequence if we take into consideration the dynamic particularization of the alveolar and maxillary skeletal and mandibular growth and development on the age groups studied.

Another interesting aspect to analyze is the *loss of both lower temporary molars* on the same quadrant. A study conducted by Leroy R. *et al* (17) in 2009, showed that at the moment of cavity deterioration of both lower temporary molars and their loss subsequent to the cavity damage, the ideal eruption sequence „3-4-5-7” is reversed, increasing the prevalence of the eruption sequence „4-3-5-7”, „4-3-7-5” and „4-5-3-7”. Thus there occurs an accelerated eruption of both premolars. This is also confirmed by us in the present study through the increase of the mean angular values between the longitudinal axis of the permanent molars and the occlusal plane close to the mean angular values of the control group, leading to their distal overhang.

The moderate correlation between the axes of first and second molars in the same quadrant suggests the fact that any molar premature loss may have an impact on the entire dento-maxillary apparatus and may have later repercussions in the development of dento-maxillary anomalies.

The theme studied by us requires further complex clinico-radiological studies

with a powerful analytical-statistical approach with a higher number of subjects, ecological repartition on genders and age stages and by using the advanced measurement techniques of CBCT type.

CONCLUSIONS

Our study proves one more time the problematic of the premature loss of lower second temporary molar, leading to a necessity to conduct more studies in this field. Its pathology fails to be limited in an intra-arch context due to the extrusion potential of second maxillary temporary molar which may generate premature contacts, occlusal interferences and may lead to the Thielemann effect on the long term (diagonal syndrome) affecting the opposite articulation.

The modification of the eruption sequence following the premature loss of temporary molars is a phenomenon which needs consideration, especially when an interceptive treatment is adopted, with the preservation of the occlusal morpho-functional complex using space maintainers.

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REFERENCES

1. Hutchinson SJ. On the Premature Extraction of the Deciduous Canines. *Br Dent Assoc J* 1884; 5:75-82.
2. Davenport IB. The Significance of the Natural Form and Arrangement of the Dental Arches of Man, with a Consideration of the Changes which Occur as a Result of their Artificial Derangement by Filling or by the Extraction of teeth. *Dent Cosmos* 1887; 29:413-439.
3. Macena MC, Tornisiello Katz CR, Heimer MV, de Oliveira e Silva JF, Costa LB. Space Changes after Premature Loss of Deciduous Molars among Brazilian Children. *Am J Orthod Dentofacial Orthop*. 2011;140(6): 771-778.
4. Zegan G. Ortodonție și Ortopedie Dento-facială, Iași, Editura Tehnopress, 2006: 44-45;
5. Lin YT, Lin WH, Lin YT. Twelve-month Space Changes after Premature Loss of a Primary Maxillary First Molar. *Int J Paediatr Dent*. 2011;21(3): 161-166.
6. Ngan P, Alkire B, Fields H jr. Management of Space Problems in the Primary and Mixed Dentition. *J Am Dent Assoc* 1999; 130:1330-1339.
7. Posen AL. The Effect of Premature Loss of Deciduous Molar on Premolar Eruption. *Angle Orthod*. 1965;35:249-251.
8. Tunison W, Flores-Mir C, Elbadrawy H, Nassar U, El-Bialy T. Dental Arch Space Changes following Premature Loss of Primary First Molars: a Systematic Review. *Pediatr Dent* 2008; 30: 297-302.
9. Hoffding J, Kisling E. Premature Loss of the Primary teeth: part I, Its Overall Effect on Occlusion and Space in the Permanent Dentition. *J Dent Child* 1978; 45: 279-283.
10. Hoffding J, Kisling E. Premature Loss of Primary Teeth: part II, The Specific Effects on Occlusion and Space in the Permanent Dentition. *J Dent Child* 1978; 45: 284-287.
11. Kisling E, Hoffding J. Premature loss of primary teeth: part III, drifting patterns for different types of teeth after loss of adjoining teeth. *J Dent Child* 1979; 46: 34-38.

12. Petcu A, Maxim A, Haba D. Correlation between the Lower First Permanent Molar Axis and the Premature Loss of Temporary Molars. *Rev Med Chir Soc Med Nat Iasi* 2009 Oct-Dec;113(4):1253-1257.
13. Cuoghi OA, Bertoz FA, Mendonça MR, Santos EC. Loss of Space and Dental Arch Length after the Loss of the Lower First Primary Molar: a Longitudinal Study. *J Clin Pediatr Dent* 1998;22:117-120.
14. Lin YT, Chang LC. Space Changes after Premature Loss of the Mandibular Primary First Molar: a Longitudinal Study. *J Clin Pediatr Dent* 1988; 22: 311– 316.
15. Kumari BP, Kumari NR. Loss of Space and Changes in the Dental Arch after Premature Loss of the Lower Primary Molar: a Longitudinal Study. *J Indian Soc Pedod Prev Dent* 2006; 24: 90–96.
16. Northway WM, Wainright RL, Demirjian A. Effects of Premature Loss of Deciduous Molars. *Angle Orthod* 1984;54:295-329.
17. Leroy R, Cecere S, Lesaffre E, Declerck D. Caries Experience in Primary Molars and its Impact on the Variability in Permanent Tooth Emergence Sequences. *J Dent*. 2009; 37(11):865-871.

NEWS

LITERATURE REVIEW CONCERNING THE LOCAL DRUG DELIVERY SYSTEMS IN THE TREATMENT OF PERIODONTITIS

In periodontitis, in order to complement non-surgical therapy, there are multiple options of antimicrobials (metronidazole, chlorhexidine, minocycline, doxycycline, tetracycline), which can be locally delivered into the mucosa. These antimicrobials are used in periodontal pockets and can inhibit or eliminate periodonto-pathogenic micro-organisms as well as modulate the inflammatory response of tissues. Currently, limited data are available regarding the relationship between effect, efficacy and clinical status of the periodontium. A review realized by a group of researchers aimed to evaluate the effect and the efficacy of different types of local drug delivery systems in clinical parameters of periodontology. Researched papers using MEDLINE via PubMed, and LILACS databases related to five types of local drug delivery systems (chlorhexidine gluconate, doxycycline hyclate, metronidazole gel, minocycline ointment and tetracycline fibers) were reviewed aiming to address the mechanism of action and the evidence of clinical effectiveness of adjunctive use of these antimicrobials following surgical and/or non-surgical therapies. Inclusion criteria defined that articles must be randomized controlled trials performed in humans and published between 1996 and 2014. The adjunctive use of local drug delivery systems with controlled release properties may provide a defined, but limited, beneficial response on periodontal pockets. Local drug delivery as an active treatment or maintenance therapy depends on clinical findings, responses to treatment described in the literature, desired clinical outcomes, and patients' dental and medical histories, including their past usage of antimicrobials (Da Rocha HA, Silva CF, Santiago FL, Martins LG, Dias PC, De Magalhães D. Local Drug Delivery Systems in the Treatment of Periodontitis: A Literature Review. *J Int Acad Periodontol*. 2015; 17 (3) : 82-90).

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COMBINED EFFECT OF THERMAL LOAD AND MECHANICAL LOAD IN TRANSTIBIAL PROSTHESIS. AN EMPIRICAL STUDY

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COMBINED EFFECT OF THERMAL LOAD AND MECHANICAL LOAD IN TRANSTIBIAL PROSTHESIS. AN EMPIRICAL STUDY (Abstract): Probably the most important factor in evaluating a patient's prosthesis is quality of life. Transtibial amputations, are among the most frequently performed major limb amputations. Many individuals with transtibial amputations successfully achieve rehabilitation at or near their preamputation levels. Discomfort in prosthetic sockets continues to be a critical challenge faced by both prosthetists and amputees. **Material and methods:** This paper proposes a fusion Graphic User Interface that combines two types of information (pressure and temperature). Data from pressure sensors and thermistors (an electrical resistor whose resistance is greatly reduced by heating, used for measurement and control) placed on the stump in transtibial prosthetics are collected in real time using a National Instruments Data Acquisition device. **Results:** All the data stored in files are available for offline processing. The user has the possibility to analyse the signal by zooming or positioning the marker and window on different parts of signal. A complex analysis that involves the pressure and temperature for a location (where both sensors are placed) is available in time domain. **Conclusions:** Blunt-prosthesis interface is characterized by few parameters among the most important are pressure and friction. The action of these parameters during static and dynamic stage is very important because their actions can produce lesions of skin at the level of interface. Despite the advancements in surgical techniques and prostheses, much still needs to be done. We made certain that the sensors were in the same location by pressing on specific cells on the residual limb during various stages of the experimentation. The highest pressures recorded were during the stance phase of walking. The curve that shows the temperature evolution or pressure in one point could differ in different points from patient to patient. **Keywords:** BLUNT, GRAFIC INTERFACE, TRANSTIBIAL PROSTHESIS, THERMISTOR, THERMAL LOAD.

The interface skin-liner has a distinctive achievement consisting in a comfortable prosthesis for a person with lower limb amputation. Amputation is a surgery that involves the section of anatomic parts: bones, muscles, blood vessels and nerves.

A study of the interface-level pressures presents a particular interest in determining the patient's comfort and quality of life (1,2). In (3) the authors propose placement of six sensors in locations chosen based on experimental criteria to cover pressure

zones for two persons with transtibial amputation. One of the main purposes is to identify areas with maximum pressure variation of these values depending on the patient's activity. A performance investigation of the patient's comfort is proposed in (4). The authors used a four F-Socket sensors array 9811 and a Prosthetic Evaluation Questionnaire which investigates patient satisfaction in relation to the two liners used (4). But from the point of view of registration equipment and pressures of the software, this solution is expensive.

In the last years, few papers discussed the influence of temperature on patient's comfort. Temperature values at skin-prosthetic interface are recorded for different points on stump and the influence of ambient temperature is also discussed. The permanent contact of inorganic materials with skin can cause cutaneous pathology (1). Recent studies reveal the fact that a 2°C increase of skin temperature in residual limb can create discomfort (2). Thermal comfort satisfaction is strongly related with ambient temperature that has influence over skin temperature, perspiration and all thermal processes in stump, skin, liner and socket (3). This study reveals that from 38 studies published in PubMed and Web of Knowledge, at least 53% of persons with amputation faced discomfort due to heat, perspiration or both inside their prosthesis, most of them having transtibial amputation. A collection of 16 thermistors and a portable signal acquisition system to record temperature curves is proposed in (4). The thermistors are placed uniformly on the stump and the evaluation of temperature evolution is made using an activity protocol of 30-minute walk bordered by 60 minutes of rest (4). A map of temperatures is presented using thermal contour plots. A new ap-

proach to record the temperature on surface of the residual limb is proposed in (5). The protocol uses Gaussian Process for Machine Learning for predictive model that has as input the liner temperature and as output the temperature of the skin located on the residual limb (5). A simple method to describe the temperature on interface skin-prosthesis is proposed in (6). The authors used 16 thermistors placed in a regular grid on stump surface to collect data. One subject was selected for test in 1h protocol rest-walk-rest. Thermal plot contour type was deduced using Matlab in an offline application. An average value of temperature over all 16 thermistors has been calculated in order to deduce the influence of thermal environment on skin-prosthesis interface (6). The experimental studies revealed the fact that a lower temperature at skin level usually reduces the patient's discomfort both due to ambient temperature and wearing of prosthesis. Few researches oriented toward thermal conductivity of materials that are used for construction of prosthesis. Thermal conductivity of materials has been calculated using Fourier's law. One of the most important conclusions of the paper is that the thermal discomfort is reduced in the case of multilayer sockets that use materials having high thermal conductivity (7). A 3-D thermal finite element model is proposed in (8). Thermal characteristics of the tissues (muscle, fat, skin, bone) and prosthetic materials were taken from literature. The skin temperature was predicted using Pennes' BHT equation of heat transfer (8). The thermal performance of prosthesis is one of the very important objectives in prosthetic design (9, 10). A socket modification, a cooling channel inserted in socket, is proposed in (9). A socket prototype was created in SolidWorks and implementation was made using a 3D printer

(9). A thermoregulatory system was proposed in (11). The authors verified the system using a phantom model, silicone liner and a thin layer on aluminium as transfer interface between thermal pump and different locations situated on phantom (11). A finite element method is used in (12). The authors propose to use finite element method in modelling temperature distribution at skin surface in order to predict it depending on external conditions. Some authors used the experimental results to design novel prostheses (13) or a complicate system for thermoregulation of temperature inside socket (14).

MATERIAL AND METHODS

A graphical interface is an interface located between the user and electronic devices such as computers, hand-held devices (mp3 players, portable media players), electronic appliances and some office equipment. To present all available information and actions, a GUI provides icons and visual indicators, in contrast to text-

based user interfaces, which provide names of commands to be typed in or text navigation. This is intended to facilitate the user's work by eliminating the process of writing the software code. For recording purposes, pressure sensors were used on type CZN-CP6 (pressure sensors) and CZN-CP9 (pressure sensors) self-adhesive wiring diagram to measure resistive input in measuring plate. An analogue digital converter used precise 14-bit resolution, and six sensors were placed in locations determined experimentally in areas with the highest solicitations (and therefore a higher pressure) according to fig. 1.

Ten thermistors of MA100GG type were placed on the sink of the stump in an experiment including a group of three patients with transtibial amputation: A1 (67 Kg, 27 years, male), B3 (30 Kg, 10 years, male) and C4 (76 kg, 32 years) (fig. 2). The thermistors are validated before using the water-bath procedure. The other patients with other diseases, e.g. diabetes, were excluded from the study.



Fig. 1. Pressure sensors



Fig. 2. Thermistor locations

Experiments were conducted by using a protocol that began with a 30-minute walk at fixed speed (a trade off between the speed's options of the two patients) and a 30-minute rest before repeating the experiment. The sensors were placed on the areas known as being the most solicited pressure locations during walk (e.g. lateral side, medial side, distal, etc.). Also, other experiments used a 2D map of the stump split in approximated rectangles with an algorithm for sensors placement in order to identify the most solicited areas by temperature evolution during walk. Finally, all the shortcomings are reflected in the patient's sensation of discomfort. In time, a combination of pressure and temperature can lead to serious affections of skin and teguments

RESULTS

A Graphic User Interface is proposed to analyse offline data collected from pressure sensors and temperature sensors. After authentication, the user can select the two options: Force (pressure) data or Temperature data. Data are collected online using a National Instruments Data Acquisition device and the files are stored in ASCII format. Data acquisition is the process of measuring an electrical or physical phenomenon such as temperature, pressure with a computer. Records for the same patient (pressure and temperature) are statistically processed. Outliers are removed and replaced by a median value, and the points outside value bordered by $\pm$ SD (standard deviation are excluded). The most probable curve is computed from the rest of the points using the LMS (Least Mean Square) method.

The interface has a module that allows calibrating the sensor, the curve being approximated by a polynomial model (a polynomial with maximum degree 7). Practi-

cally, the coefficients can be stored individually for each sensor or for all sensors for future purpose. The curve that shows the temperature evolution or pressure in one point could differ in different points from patient to patient.

Probably the most important factor in evaluating a patient's prosthesis is Quality of Life. The most common evaluation of this factor uses questionnaires, the Prosthetic Evaluation Questionnaire being one of the most used in practice (15). Prosthetic Evaluation Questionnaire was developed to provide quantitative measures for patient's satisfaction using 54 qualitative questions. One of the most common materials was used for all patients' prostheses: socket – PP/PE (90% Polypropylene, 10% ethylene), Young's module = 1.5 GPa, Poisson coefficient = 0.3 and liner - soft Pelite (is a polyethylene closed cell foam commonly used as an interface material in prosthetics), with Young's module = 0.38 MPa, Poisson coefficient = 0.39. In this stage of research, we want to measure the degree of comfort using Prosthetic Evaluation Questionnaire for all three patients. Average empiric coefficients were set for fused data, pressure and temperature ($\alpha = 0.9$ for pressure and 0.1 for temperature). The final Prosthetic Evaluation Questionnaire was found to be 43, 56, and 38 for three patients, which suggests that it is possible for some pairs of socket-liner materials not to be very adequate for some patients. These values are suggested by the fast variation in force versus time (5-6 sec) and a very slow modification in temperature in the same time interval, constant in practice. Further investigation is needed, using new models which study how temperature can affect the patient's discomfort sensation caused by a pressure (force) on the skin at skin-liner interface.

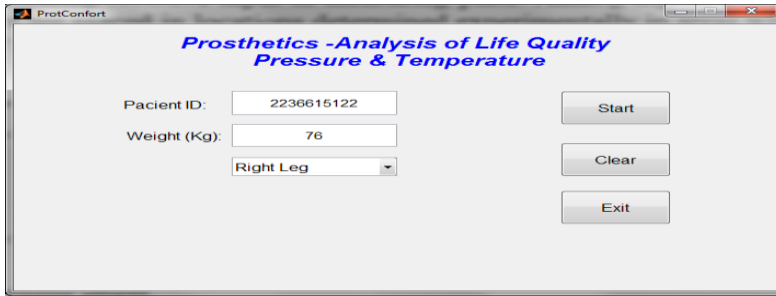


Fig. 3. First page of program after log-in.

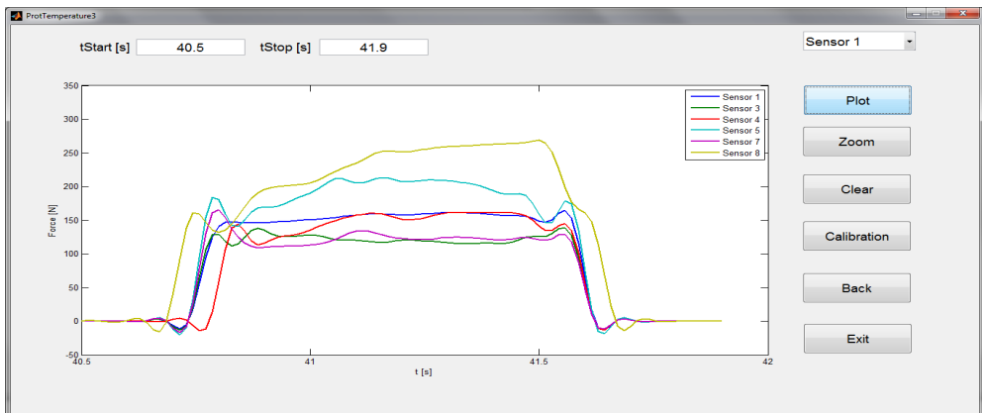


Fig. 4. Record of an episode of movement (walk, last 2 seconds).

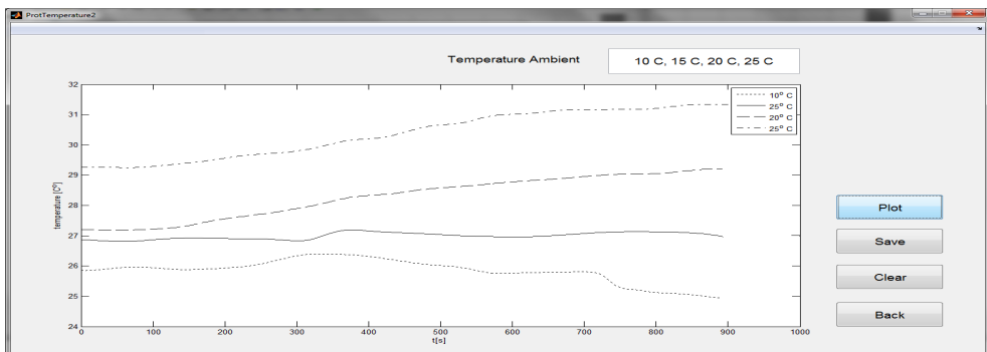


Fig. 5. Wave form temperatures, sensor 3, pos. 5, medial position for ambient temperature 10° C, 15° C, 20° C, and 25° C.

CONCLUSIONS

In this article, Graphic User Interface may contribute towards improvement in the quality of socket design for amputee pa-

tients. This interface has been the most common computer modelling method used to try to predict stresses at the socket interface. The contact zone between two

surfaces generates two types of friction, static friction and kinetic friction. The quality and comfort of a prosthetic socket can determine the daily duration for which patients use their artificial limbs. More studies are necessary to find a relationship between patient's comfort sensation and

materials used in prosthetic. Beside a custom approach for each patient, statistic studies need to include other factors, such as age or sex. It is clear that a large group of subjects and medical validation are necessary before these preliminary results become applicable.

REFERENCES

1. Dillingham TR, Pezzin LE, Mackenzie LE, Burgess AR. Use and satisfaction with prosthetic devices among persons with trauma-related amputations: A long-term outcome study, *AM J Phys Med Rehabil* 2001, 80: 563-571.
2. Yeung LF, Leung AKL, Zhang M, Lee WCC. Effects of long distance walking on socket-limb interface pressure, tactile sensitivity and subjective perceptions of transtibial amputees, *Disab Rehabil* 2013,35(11): 888-893.
3. Sanders JE, Lam D, Dralle AJ, Okumura R. Interface pressures and shear stresses at thirteen socket sites on two persons with transtibial amputation, *J Rehabil Res Develop* 1997, 34(1): 19–43.
4. Ali S. Clinical investigation of the interface pressure in the transtibial socket with Dermo and Seal-In X5 liner during walking and their effect on patient satisfaction, *Clin Biomech* 2012, 27: 943–948.
5. Van De Weg FB , Van der Windt DAWM. A questionnaire survey of the effect of different interface types on patient satisfaction and perceived problems among transtibial amputees, *Prosthet Orthot Int* 2005, 29(3): 231-239.
6. Peery JT , Ledoux WR, Klute GK. Residual-limb skin temperature in transtibial sockets, *J Rehabil Res Develop* 2005, 42(2): 147-154.
7. Ghoseiri K, Safari MR. Prevalence of heat and perspiration discomfort inside prostheses. Literature review, *J Rehabil Res Develop* 2014, 51(6): 855 - 868.
8. Klute, GK, Huff E, Ledoux WR. Does Activity Affect Residual Limb Skin Temperatures, *Clin Orthop Relat Res* 2014, 472: 3062–3067.
9. Mathur N, Glesk I. Skin Temperature Prediction in Lower Limb Prostheses, *IEEE J Biomed Health Inform* 2014,20:134-149.
10. Huff EA, Ledoux, WR, Berge JS, Klute GK. Measuring Residual Limb Skin Temperatures at the Skin-Prosthesis Interface, *JPO* 2008, 20(4): 170-173.
11. Klute GK, Rowe G, Mamishev AV, Ledoux WR. The thermal conductivity of prosthetic sockets and liner, *Prosthet Orthot Int* 2007, 31(3): 292-299.
12. Peery JT, Klute GK, Blevins JJ, Ledoux WR. A Three-Dimensional Finite Element Model of the Transibial Residual Limb and Prosthetic Socket to Predict Skin Temperatures, *IEEE Transact Neural Syst Rehabil Engin* 2006, 14(3): 336-343.
13. Webber CM, Davis BL. Design of a novel prosthetic socket: Assessment of the thermal performance, *J Biomech* 2015, 48: 1294-1299.
14. Ali S. Clinical investigation of the interface pressure in the transtibial socket with Dermo and Seal-in X5 liner during walking and their effect on patient satisfaction, *Clin Biomech* 2012, 27: 943-948.
15. Legro MW. Prosthesis evaluation questionnaire for persons with lower limb amputations: assessing prosthesis-related quality of life, *Arch. Phys Med Rehabil* 1998, 79: 931-938.

EVOLUTION OF SYNCHRONOUS BILATERAL BREAST CARCINOMA IN A YOUNG PATIENT

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EVOLUTION OF SYNCHRONOUS BILATERAL BREAST CARCINOMA IN A YOUNG PATIENT (Abstract) Bilateral breast cancer incidence is appreciated to be between 0.3 to 12% and is determined either by a hereditary load associated with chromosomal instability under the effect of environmental factors, or by the evolution in a particular hormonal context which gives biological aggressiveness. We present the case of a patient, aged 38 years, clinically, imagistic and bioptic diagnosed with left axillary lymph node metastases of breast carcinoma NST invasive G3, IHC - RE = 60%, RP = 30%, HER2neu = 2 +, Ki67 = 20% , in August 2013. Patient followed neoadjuvant chemotherapy treatment during September-October 2013. In December 2013 she was clinically and imaging diagnosed with bilateral breast cancer, for which surgical intervention was done which consisted of bilateral radical Madden mastectomy with bilateral axillary lymphadenectomy. BAP - invasive carcinoma NST: left breast - pT2mN3a G2, right breast - pT3mN3a G2, IHC - RE = 90%, RP = 70% HER2neu = 2 +, Ki67 = 50%. During the period of January-March 2014, the patient followed adjuvant chemotherapy and Herceptin. Bilateral breast ultrasound assessment in April 2014 revealed: left axilla - liquid blade 29 / 6mm; right axilla - oval ganglion 9 / 5mm. Abdominal and pelvic ultrasound: empty uterine cavity, bosselated contour; at left ovary level multiple cystic formations. During the period of May-June 2014, adjuvant radiation therapy and ovarian irradiation was administered to the patient. Subsequently hormone therapy was initiated. Following CHT / ovarian irradiation patient continues to experience intermittent uterine bleeding, which is why a total hysterectomy with bilateral ovariectomy was done, and BAP: cervical, endometrial and left ovary with tumor multifocal infiltration with histopathological aspect of invasive breast carcinoma NST. Periodic imaging evaluations do not reveal local or distant recurrence. The particularity of this case is synchronous bilateral breast cancer diagnosis in a young patient complicated in its evolution by ovarian metastases. This form of metastasis is rare in young women and occurs in advanced stages of the disease. **Keywords:** SYNCHRONOUS BILATERAL BREAST CARCINOMA, MULTIMODAL TREATMENT, OVARIAN METASTASES.

Breast cancer is the most common type of cancer among women with increased incidence due to screening, resulting in improved prognosis and survival [1, 2]. In breast neoplasm, clinical forms and biolog-

ical characteristics are very diverse. Of all breast cancers, BBC (bilateral breast cancer) is 2-6% [1]. Bilateral breast cancer incidence is appreciated to be between 0.3 to 12%. In breast cancer the most common

secondary dissemination are of the bone, liver, lung and brain, but may be metastatic sites other organs such as the peritoneum, ovaries and teguments.

MATERIAL AND METHOD:

In this paper we present the case of a patient, aged 38 years, clinically, imagistic and bioptic diagnosed with left axillary lymph node metastases of mammary gland carcinoma NST invasive G3, IHC (immunohistochemistry) ER (estrogen receptor) = 60%, PR (progesterone receptor) = 30%, Her 2neu = 2 +, Ki67 = 20%, in August 2013. Neoadjuvant chemotherapy followed during September-October 2013, 3 CAF protocol cycles (cyclophosphamide 800mg, doxorubicin 80mg, 5-FU 800mg).

In December 2013, a bilateral breast ultrasound detected in the right breast a nodular formation of about 1.5 - 2cm, while on the left breast endured placard and bilateral axillary infracentrimetrical adenopathy.

A pelvic ultrasound from December 2013 detects a heterogeneous consistence uterus, 45/36 / 72mm with two fibroid nodules 19 / 15.5mm and 12 / 15mm with 5.7mm endometrium, left ovary 40 / 24mm positioned retrouterin, normal consistency, right ovary 32 / 17mm normal echo-structure and empty Douglas space.

A bilateral radical mastectomy type Madden with axillary bilateral lymphadenectomy with BAP (ID pathological) - NST invasive carcinoma: left breast - pT2mN3a G2, 11 excised lymph node metastasis; right breast - pT3mN3a G2, 15 lymph node metastasis in 19 excised IHC- RE = 90%, RP = 70%, Her 2neu = 2 +, Ki67 = 50% , FISH: HER2neu amplified. During the time period January-March 2014, the patient followed adjuvant chemotherapy: 1 CAF protocol cycle, 4 series of chemotherapy with Paclitaxel 300mg and initiated therapy with 300mg of Herceptin.

In April 2014, imaging evaluation through bilateral breast ultrasound revealed: left axillary - liquid blade 29 / 6mm; right axillary - oval ganglion 9 / 5mm. Abdominal and pelvic ultrasound: empty uterine cavity, bosselated contour; at left ovary level multiple cystic formations.

During May-June 2014 was administered to the patient adjuvant radiotherapy at ACC ClinaciX10, photons E = 10MV, DT = 42.56Gy/16fr/2.66Gy/fr/ chest wall bilaterally and axillary lymph nodes areas, bilateral supraclavicular region and right lateral-cervical (Fig.1, Fig.2) and ovarian irradiation at Clinac accelerator 2100sc, with photons E = 10MV, DT = 12Gy/4fr/3Gy /fr. Subsequently adjuvant hormone therapy was initiated with Tamoxifen.



Fig. 1. Treatment plan: a- target volumes delineation; a, b – patient in simulation position

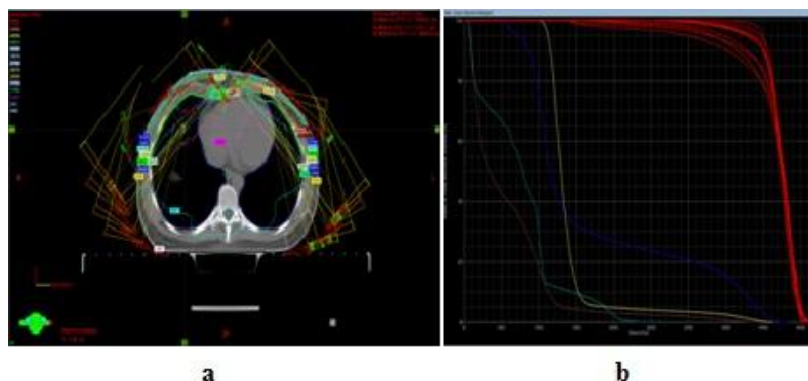


Fig. 2 Treatment plan: a- radiation fields; b- dose-volume histogram evaluation

In the context of abdominal and pelvic ultrasound appearance and persistent uterine bleeding even after ovarian suppression by chemotherapy and radiation therapy, total hysterectomy with bilateral ovariectomy was performed. BAP: cervical, endometrial and left ovary with tumor multifocal infiltration with histopathological aspect of invasive breast carcinoma NST.

Thorax CT (computer tomography) in September 2015 - without signs of local or distant recurrence, persistence of microadenopathies of $\sim 9 / 10\text{mm}$ right hilar. In upper abdominal sections a simple hepatic cyst was identified in the segment IVb of $\sim 4.5\text{mm}$.

Pelvis MRI (magnetic resonance imaging) in November 2015 – total hysterectomy with bilateral ovariectomy without pathological contrast intake – stationery aspect, absence pelvic adenopathies.

DISCUSSION:

The risk of developing BBC is two to six times higher in women with history of unilateral breast cancer [2, 3].

BBC can be classified into subcategories, synchronous and metachronous. This classification is based on the time interval

between diagnosis of primary tumor in the first breast and the second breast. Some previous studies have defined the synchronous bilateral breast cancer (SBBC) as the development of breast cancer in the time interval of 0- 12 months [4].

According to the WHO guidelines in 2012, BBC is defined as SBBC when contralateral breast carcinoma is diagnosed within three months [1].

SBBC is defined in some studies, from an epidemiological point of view, as the development of breast cancer within 12 months [5,6]. The SBBC incidence is relatively low, between 1 and 3% of all breast cancers. SBBC and MBBC have different biological characteristics, reflected in the histopathological characteristics, stage and prognosis [7].

The appearance of the second contralateral breast tumors can be synchronous (in less than 6 months after the first primary tumor in the first breast) or metachronous (after 6 months of diagnosis of the primary tumor in the first breast) [8].

In the presented patient case, the contralateral breast cancer was diagnosed 5 months after the left breast cancer diagnosis and was considered synchronous, ac-

cording to the interval of less than 6 months.

Some studies have indicated a worse prognosis and low survival rate for BBC. Conversely, other studies have claimed that there is no significant difference in the survival rate between UBC and BBC. Despite previous and ongoing studies, the prognosis and survival of patients with BBC remain uncertain [1].

Synchronicity and metachronicity are usually associated with local recurrence, hematologic and lymphatic spread to the lungs, bones and liver.

Early detection of contralateral breast cancer is of the most importance, emphasizing the importance of breast self-examination [9]. The standard for breast cancer screening is the mammography, some authors recommend breast MRI scan, which is more sensitive in comparison to mammography, but the high cost and lack of availability is limiting as a screening indication. There are no clear treatment guidelines for bilateral breast cancer. Patients are often treated with bilateral mastectomy, conservative surgery has an unclear importance [9].

In our case, multimodal treatment was administered according to the stage, histological and prognostic factors individually for each of the tumors.

Studies suggest that there is no significant difference in survival rate for patients with MBBC compared with those with SBBC. Synchronous tumors were associated with decreased survival in comparison to metachronous tumors [10].

Most often, patients with BBC are receiving radical surgery based on the idea that these tumors are biologically aggressive. However, scientific evidence to support this are split. Conservative treatment

for BBC may be feasible also in the case of unilateral breast cancer [11].

BBC is more common in young aged women and most of these tumors are estrogen dependent. There is a concordance between the primary tumor and contralateral breast cancer from a histological point of view, of the receptors ER, PR and HER2. Some studies argue that there is no clear relationship between positive ER and PR and tumor bilateral localization. It seems that the BBC incidence is higher in cases with the overexpression HER-2 [2].

BC can metastasize to the bone, liver, lung and brain, but can affect other organs such as the peritoneum, ovaries and tegument.

Metastatic ovarian tumors, that are called Krukenberg tumors, occur frequently after the age of 40 years [12]. The appearance of these tumors at a young age is very rare and represents approximately 2% of all cases. The occurrence of ovarian metastases in breast cancer represents an advanced stage of the disease [13].

It seems that patients with Krukenberg tumors with BC as a starting point, unlike other tumor locations, have a good prognosis [13]. Surgery is the optimal treatment for these tumors, as it has been practiced in this case.

CONCLUSIONS:

The particularity of this case is synchronous bilateral breast cancer diagnosis in a young patient complicated in its evolution with ovarian metastases. This form of metastasize is rare in young women and occurs in the advanced stages of the disease. The prognosis for this patient is reserved considering the age under 40 years, synchronous neoplasia, nodal status (pN3a bilateral), the advanced stage (IIIC), Her

2neu positive status.

In the case of synchronous bilateral breast carcinoma there should be applied

adequate multidisciplinary management and rigorous supervision of patient to improve survival and quality of life.

REFERENCES

1. Eliyatkin N, Zengel B, Yagci A, et al. Properties of Synchronous Versus Metachronous Bilateral Breast Carcinoma with Long Time Follow Up. *Asian Pac J Cancer Prev*. 2015; 16(12): 4921-6
2. Hartman M, Czene K et al. Incidence and prognosis of synchronous and metachronous bilateral breast cancer. *J Clin Oncol*, 2007;25, 4210-6.
3. Chen Y, Thompson W et al. Epidemiology of contralateral breast cancer. *Cancer Epidemiol Biomarkers Prev*, 1999;8, 855-61.
4. Chen SF, Du CW et al. The molecular and clinicopathologic characteristics of bilateral breast cancer. *Sci Rep*, 2013;3, 2590.
5. Huo D, Melkonian S et al. Concordance in histological and biological parameters between first and second primary breast cancers. *Cancer*, 2011; 117, 907-15.
6. Ibrahim NY, Sroor MY et al. Impact of bilateral breast cancer on prognosis: synchronous versus metachronous tumors. *Asian Pac J Cancer Prev*, 2015;16, 1007-10.
7. Holm M, Tjonneland A et al. Prognosis of synchronous bilateral breast cancer: a review and meta-analysis of observational studies. *Breast Cancer Res Treat*, 2014;146, 461-75.
8. Londero A., Bernardi S., et al. Synchronous and Metachronous Breast Malignancies: A Cross-Sectional Retrospective Study and Review of the Literature, *BioMed Research International* Volume 2014 (2014), Article ID 250727, doi: 10.1155/2014/250727.
9. Kappikeri VK, KriplaniAM. Bilateral synchronous carcinoma breast- a rare case presentation. *Springerplus*. 2015 Apr 22;4:193. doi: 10.1186/s40064-015-0953-3. eCollection2015.
10. Branica BV, JezekSS et al. Synchronous bilateral breast carcinoma with two different morphology subtypes: A case report., *Coll Antropol* 2011; 34:701–704.
11. Padmanabhan N, Subramanyan A et al. Synchronous Bilateral Breast Cancers. *J Clin Diagn Res*. 2015 Sep;9(9):XC05-XC08. doi: 10.7860/JCDR/2015/14880.6511. Epub 2015 Sep
12. Hatwal D, Joshi C et al. Krukenberg tumor in a young woman: a rare presentation. *Indian J Pathol-Microbiol*. 2014 Jan-Mar;57(1):124-6. doi: 10.4103/0377-4929.130919.
13. Jiang R, Tang J et al. Surgical treatment for patients with different origins of Krukenberg tumors: outcomes and prognostic factors. *Eur J SurgOncol*. 2009 Jan;35(1):92-7. doi: 10.1016/j.ejso.2008.05.006. Epub 2008 Jul 16.

THERMOPLASTIC MATERIALS APPLICATIONS IN RADIATION THERAPY

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THERMOPLASTIC MATERIALS APPLICATIONS IN RADIATION THERAPY (Abstract)

This is an example of the use of thermoplastic materials in a high-tech medicine field, oncology radiation therapy, in order to produce the rigid masks for positioning and immobilization of the patient during simulation of the treatment procedure, the imaging verification of position and administration of the indicated radiation dose. Implementation of modern techniques of radiation therapy is possible only if provided with performant equipment (CT simulators, linear accelerators of high energy particles provided with multilamellar collimators and imaging verification systems) and accessories that increase the precision of the treatment (special supports for head-neck, thorax, pelvis, head-neck and thorax immobilization masks, compensating materials like bolus type material). The paper illustrates the main steps in modern radiation therapy service and argues the role of thermoplastics in reducing daily patient positioning errors during treatment. As part of quality assurance of irradiation procedure, using a rigid mask is mandatory when applying 3D conformal radiation therapy techniques, radiation therapy with intensity modulated radiation or rotational techniques. Key words: IMMOBILIZATION, THERMOPLASTIC MASKS, RADIOTHERAPY.

This article presents one of the uses of thermoplastic materials in high-energy radiation treatment of malignant neoplasms, produced by linear particles accelerators (radiation).

Modern techniques of external radiation therapy allow high doses of X-rays or electrons in target tumor volumes defined after adapted localization and tumor extension criteria, reducing radiation doses to organs at risk in the immediate vicinity of the tumor.

These complex treatments involve accessories to immobilize the patient, high performance imaging methods: computer

tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) for the precise localization of the tumor volume, algorithms that also include tissue inhomogeneities, high performance accelerators with multilamellar collimators, 3D conformal treatment, treatment with intensity modulated radiation therapy (IMRT), rotational therapy and imaging system for checking the treatment position of the patient [1].

MATERIAL AND METHOD:

Steps to follow in radiation therapy

1. CT Simulation. Carrying out an ac-

curate CT simulation by proper preparing and positioning the patient and through use of means of immobilization that allow further daily reproducibility of the position during the 5-7 weeks of treatment, is one of the most important steps for a proper treatment [2].

Depending on the anatomical region where the tumor that will be subjected to radiation is, the patient is comfortably positioned on a special support placed on the table of the simulator, afterwards the patient is immobilized in this position with the use of masks obtained by soaking for a few minutes (the time depends on the composition and thickness of the material) at 75°C thermoplastic plates, of adequate size for the immobilized area. On the external surface of the formed mask are placed radiopaque markers corresponding to the

cutaneous projection of the origin point for the CT scanning. After completion of the simulation, on the mask are noted patient identification and accelerator data that will be used for treatment and the mask will be handed to the assistant from the accelerator (fig. 1).

In case that during treatment the patient undergoes significant changes regarding the immobilized area (weight loss, reduction of initially voluminous adenopathy or local edema), leading to a looser mask or, conversely, to a tighter mask, the patient undergoes a process of resimulation and immobilized with a mask molded according the new aspect of the area of interest [3].

For preventing local changes in the pre-treatment phase it is recommended that the time from the CT simulation to onset of treatment does not exceed two weeks.

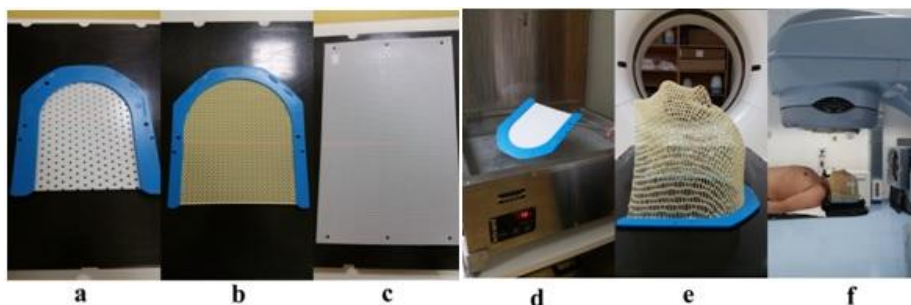


Fig. 1. Examples of the thermoplastic material prior to molding (1a, 1b, 1c), modeling in tank at 75°C (1d) and after molding (1e, 1f).

2. The treatment plan. The development of the treatment plan is based on shaping the target volumes and organs at risk on CT images obtained from simulation, followed by the prescription of the dose to the target volume and dose constraints for organs at risk.

The following volumes are identified: tumor macroscopic volume - GTV (gross tumor volume), the volume that estimates the microscopic extensions, the area of

tumoral risk - CTV (clinical target volume) and target volume planned, which takes into account movement and positioning errors - PTV (planning target volume). The edges that are added to PTV depend on the irradiated zone, availability and quality of immobilizers used, the frequency of imaging verifications of the treatment position (daily, weekly), the average errors recorded in each radiation laboratory.

Completion of the treatment plan (fig.

2) involves the use of algorithms that simulate the effect of passing through of the beams of radiation through the target vol-

ume and generates the characteristic izo-doses as well as dose-volume histograms [4].

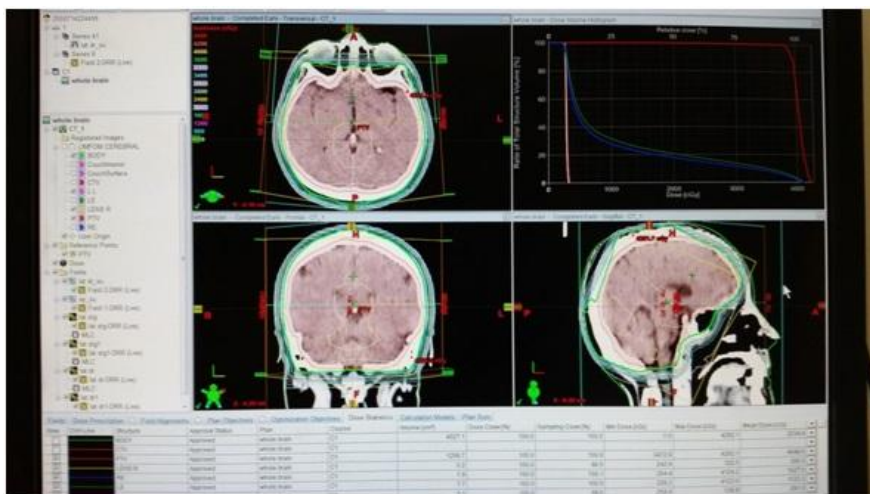


Fig. 2. The treatment plan for a patient with primitive brain lymphoma

3. Controls and implementation of treatment. The administration of high total radiation doses (50-70Gy) in tumor volume is done fractionated, with daily doses of 1,8-2Gy during 5-7 weeks, which involves repeating the verification procedures daily.

Positioning errors are verified through imaging by overlapping anatomic landmarks contoured in the images from the simulation with landmarks from the control images on the accelerator table (fig. 3) and are corrected before each treatment session [5].

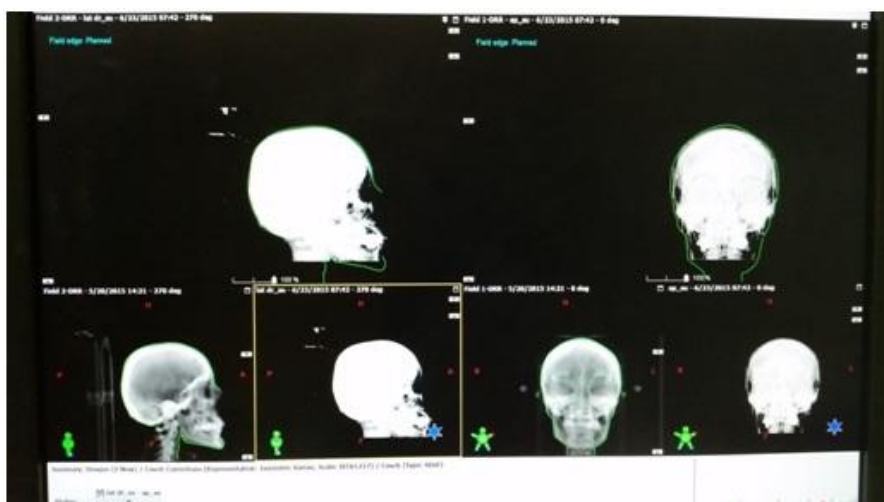


Fig. 3. Imaging treatment position control, achieved at the accelerator

DISCUSSIONS

The use of thermoplastic masks for patients treated in the Radiation Laboratory of Regional Oncology Institute, Iasi has become a common practice, especially for irradiation of primitive or secondary brain tumors and also head and neck tumors. Availability of immobilization systems has reduced positioning errors (average errors 1-2mm corrected prior to administration of radiation dose) and enables advanced radia-

tion techniques (IMRT, RapidArc).

CONCLUSIONS

This is a model of practice for accurate irradiation of tumor target volume, while protecting adjacent critical structures, which demonstrates the importance of patient immobilization for treatment reproducibility and safety, widely recognized and which continuously raises concerns to optimize immobilizers [6].

REFERENCES

1. Hoskin P., Radiotherapy in Practice: External Beam Therapy, Second Edition (2012).
2. Mutic S, Palta JR., et al., Quality assurance for computed-tomography simulators and the computed tomography-simulation process: Report of the AAPM Radiation Therapy Committee Task Group No.66. *Medical Physics* 2003;30(10): 2762-92.
3. Boda-Heggemann J., Walter C., Rahn A. *et al.*, Repositioning accuracy of two different mask systems-3D revisited: comparison using true 3D/3D matching with cone-beam CT. *Int. J. Radiat. Oncol. Biol. Phys.*, 2006; 66(5): 1568-1575.
4. Klein EE., Hanley J., Bayouth J., *et al.*, AAPM Task Group 142 report: Quality assurance of medical accelerators, *Medical Physics* 2009; 36(9): 4197-216.
5. Vieira SC., Dirkx ML., Pasma KL., Heijmen BJ., Fast and accurate leaf verification for dynamic multileaf collimation using an electronic portal imaging device. *Medical Physics* 2002; 29(9): 2034-40.
6. Fuss M., Salter BJ., CheeK D., Sadeghi A., Hevezi JM., HermanTS. Repositioning accuracy of a commercially available thermoplastic mask system. *Radiother. Oncol.* 2004; 71(3): 339-345.

A RENAISSANCE PROMOTER OF MODERN SURGERY

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A RENAISSANCE PROMOTER OF MODERN SURGERY (Abstract): The present paper aims, exploring the history of Renaissance medicine, to evoke the figure and work of the priest, surgeon and anatomist, Guido Guidi (Vidus Vidius) (1509-1569). The XVIth century is considered a period marked by artistic and scientific effervescence in the western part of Europe and Guido Guidi was a first order personality, grandson of Domenico Ghirlandaio and friend of Benvenuto Cellini. He was appointed by the King Francis I the first professor of anatomy and surgery at the newly founded Collège de France. On demand of the King, he wrote *Chirurgia è Graeco in Latinum conversa Vido Vido Florentino interprete, cum nonnullis eiusdem Vidii comentariis* (1544), a beautifully illustrated original surgery book that became for the following two centuries the main source in teaching surgery. Our study realized a detailed assessment of the book and especially of its illustrations belonging to Francesco Salviati. Exploring the life of Guido Guidi, we were also able to point out other significant contributions in the field of anatomy and clinical medicine as *De anatome* the first book where are presented disarticulated, the bones of the skull base and also the discovery of the chickenpox. Some surgical personalities attributed to him both the elaboration of the term *appendix vermiformis* and the first description of an aneurysm, he treated with the help of Fallopio. Although forgotten today, Guido Guidi was a leading figure of the Renaissance medicine both in France and Italy. **Keywords:** GUIDI, SURGERY BOOKS, RENAISSANCE MEDICINE, SALVIATI, COLLEGE DE FRANCE.

At the beginning of the XVIth century Florence was the theater of major social and political convulsions occurring against the background of the economic prosperity and artistic effervescence at the peak of the Renaissance. The richness and patronage of the Medici family decisively contributed to the development of an unequaled Florentine artistic universe and to the apparition of brilliant masters and apprentices who scien-

tifically studied the human body through dissection in order to achieve a realistic representation in works of art. This was the moment when the canons of anatomical drawing were established and the artistic anatomy began to be taught by cadaver dissection at the Arcispedale Santa Maria Nuova. Here Leonardo da Vinci (1452-1519) and Marcantonio della Torre (1481-1511) started to write a book with drawings

after dissection preparations but the plague of Florence (1511) interrupted this collaboration, due to the death of della Torre.

In 1540 was established, under the patronage of Grand Duke Cosimo I de Medici, the "Accademia degli Umidi", which later became "Accademia Fiorentina" (in 1541) (1). Here, anatomy was thought initially by Cosimo Roselli (1439-1507) and later by his pupils, Piero di Cosimo (1462-1521), Andrea del Sarto (1486-1531), Domenico Ghirlandaio (1449-1494). This effervescent atmosphere marked by the enthusiastic desire for knowledge, developed the brilliant triumvirate Leonardo da Vinci, Michelangelo (1475-1564) and Benvenuto Cellini (1500-1571).

LIFE AND ACTIVITY

In this artistic Renaissance Florence was born, on February 10, 1509 (2, 3, 4). Guido Guidi, the son of the well-known Florentine physician Giuliano Guidi and Costanza Bigordi, daughter from a second marriage of the great painter Domenico Ghirlandaio.

The data of Guido Guidi's childhood and how he obtained his university degrees are missing (5), but it is believed that the young man, with artistic blood in his veins and raised by his father in the medical tradition required by the Hippocratic oath, received a humanist education of the highest quality. In the first phase of his life, until 1535, he studied in Florence, becoming a connoisseur of Greek and Latin. Given his high education and early erudition he was noticed by the Cardinal Ridolfi, who took him under his protection. In the Cardinal's entourage, the young Guidi had access to the famous Laurentian library that housed manuscripts of great value in all areas of science and art. Among them there was a codex of the Byzantine physician

Nicetus from the X-th century, which contained, collected by Apollonius of Kitium, the Hippocratic works: *De ulceribus*, *De fistulis*, *De vulneribus capitis*, *De fracturis*, *De articulis* and *De officina medicinae*, the last three commented by Galen, in Galen's treatise *De fasciis* and Oribasius, *The laqueis and De machinamentes*.

Between 1535 and 1538, Guido Guidi went to Rome where he worked with famous surgeons of the time, Antonio Ponzanello and Giacomo Rastelli, the latter being the personal surgeon of Popes Clement VII and Paul III. Under the guidance of Roman masters he became familiar with the use of contention and continuous extension devices in the treatment of strains and fractures. It seems that in Rome Guido Guidi met for the first time Benvenuto Cellini and warm friendship developed between the two Great Men.

After returning to Florence, Cardinal Ridolfi encouraged Guido Guidi to translate *De ulceribus* and *De fistulis*, adding not only his own comments but also that of Aristotle (*causam nos docuit Aristoteles, quum tradit calorem in unu coactum augeri..*) and of Eudemus (370-300 BC), Cornelius Celsus (25 BC-50 AD), Paulus in Aegineta (625-690), and Soranus of Ephesus (ca. AD 98-138) (*Soranus, cuius scripta de vulneribus capitis adhuc extat...*) as is specified in *Chirurgia* pp. 67-72.

King Francis I of France informed by Primaticcio (1504 - 1570), by the diplomat Alamanni and by Cardinal Ridolfi himself about the audacious attempt of the young erudite, invited him to Paris in 1542. From that moment the life of Guido Guidi had sensationally changed. He presented to the King the original codex and his translation of it, making an incredible impression. He was appointed the first professor of medicine and surgery at the newly established

Collège de France, the personal physician of the King, and was requested to complete the translations in order to publish a book of surgery, according him important ecclesiastical benefits.

At Paris, where Guidi taught and practiced medicine between 1542-1547, for unknown reasons he changed his name, becoming Vidus Vidius as he will be cited in the classical anatomical literature, including *Nomina Anatomica*. We believe that the truth is more subtle; tempted by the advantages of belonging to the clerical elite, Guido Guidi, skillful user of Latin, Greek and Arabic, had chosen for himself this cognomen derived from "widower" or "alone", thus declaring his intention of

becoming a priest. This idea is supported by at least two arguments: Tuscan contemporaries, including Cellini, continued to call him Messer Guido Guidi; in a pirate edition of *Chirurgia è Graeco in Latinum conversa* from 1553, the author was named Guy i.e. Guido the Widower (6).

In his private life, fate favored him again. The young Florentine became very good friend with Benvenuto Cellini who hosted him in his castle, Petit Nesle and, in his autobiography, made him an extremely flattering characterization. Here he met Pierre Galtier, the future editor of *Chirurgia è Graeco in Latinum conversa*, who worked in typography in the Paris castle of Benvenuto Cellini.

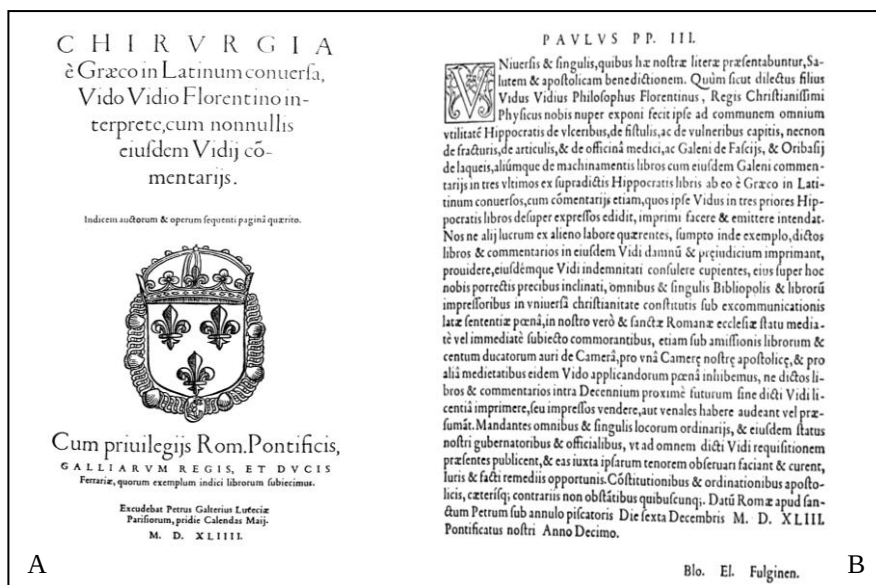


Fig. 1. A. Main opening page of *Chirurgia è Graeco in Latinum conversa*, decorated with the royal crown with the lilies of France. B. The papal prohibition against copying, reproducing or republishing the book (copyright) (7).

Guido Guidi became the first professor of medicine at the Collège de France, his clear and attractive public lectures on medicine and surgery, as well as his elegant

style (also reflected in his writings) brought new students and a large audience and at their request he began to carry out public demonstrations of anatomy. He worked

intensively until 1543 completing (fig.1) the book entitled *Chirurgia e Graeco in Latinum conversa*, Vido Vidio Florentino, cum nonnullus eiusdem Vidii comentariis (7).

It seems that the book was printed under

the guidance of Benvenuto Cellini himself, as demonstrated by the analysis of the capital letters at the beginning of the chapters, in which can be seen the floral ornaments used by Cellini for the armor of Cosimo I de' Medici (8) (fig. 2).



Fig. 2. Capital letter from *Chirurgia è Graeco in Latinum conversa*, decorated with floral motifs that are found in Benvenuto Cellini, on the bronze bust of Cosimo de' Medici, Museo Nazionale del Bargello in Florence (7, 20).

Guidi presented the book to the King Francis I, requiring a protection against plagiarism and republication by others. The King wrote him an eulogistic foreword, calling him "*notre cher & bien ayme maistre Vidus Vidius de Florence, docteur en medecine, notre medecin ordinaire & lecteur en Paris*" and then he added "*nous a faict dire & remostrer, quil estoit apres avoir laisser a imprimer a ses grandz fiz, mises & depenz, & au gand proffit & promotion des estudians en medecine & principalement en la chirurgie ung liure intitule....pour le teps & terme de six ans prochainement venant a commencer du jour & date de ces presentes, autres imprimeurs ne puissent imprimer ne vendre ledict liure ensemble, ou par ses parties, que ceulx a qui ledict permectra*" establishing penalties and significant punishments for infringement of that prohibition. The royal document dated January 2, 1543 (7, p. 22 iij) is the first known written copyright. But the

author was not satisfied with that. He presented the manuscript to Pope Paul III and received, after 10 months of thorough review of the book in the papal chancery, a brief preface that proclaimed the originality and usefulness of the book and prohibited the partial or total reproduction under the penalty of excommunication (7, p. 22 ij).

Chirurgia è Graeco in Latinum conversa that became a reference book for the study of surgery in the XVI to XVIII centuries, begins as a result of author's erudition, with a bilingual alphabetical index in Greek and Latin, where, across 14 pages, the correspondence of the surgery terminology is specified in both languages. Moreover, Guidi presents an index of authors and works, outlining that the first six chapters, *De ulceribus*, *De fistulis*, *De vulneris capitis*, *De fracturis*, *De articulis*, *De officina doctors*, belong to Hippocrates and are commented by Vidius (the first three chapters) and the following three chapters to Galen.

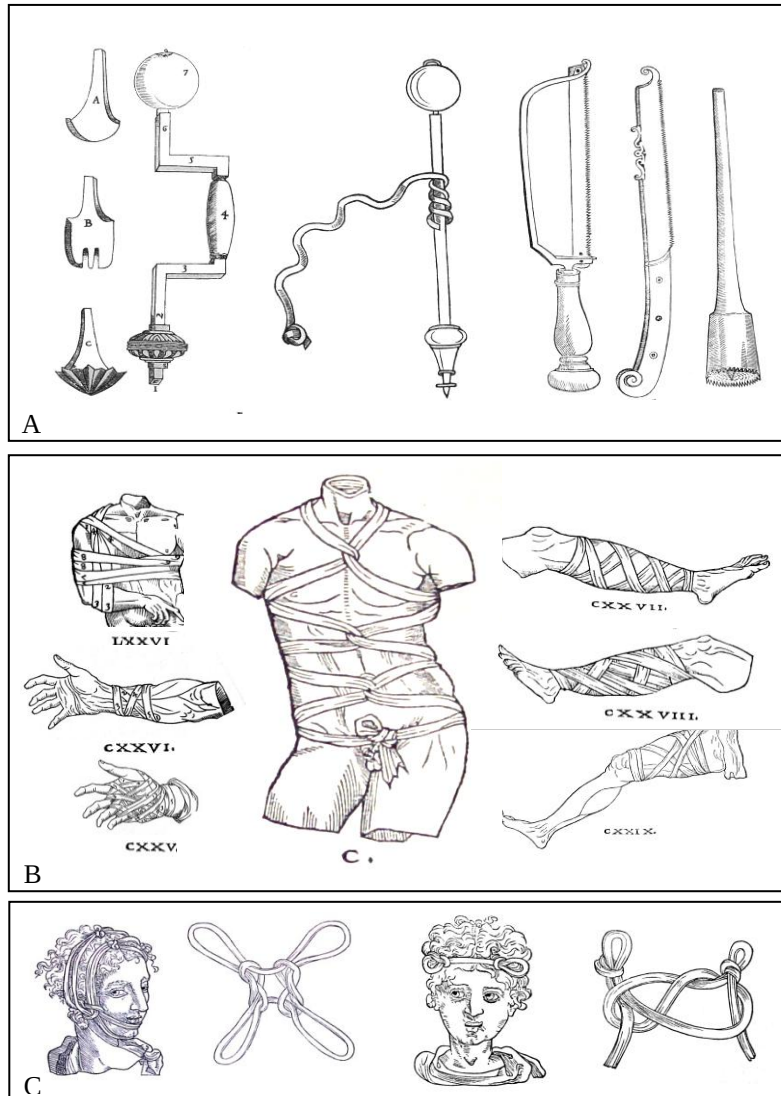


Fig. 3. A. The instruments imaged for different interventions on the bones (commentary illustrating *De fracturis*); B. Types of bandages for limbs and trunk; C. Models of surgical knots (7).

Chapter VII contains Galen's *De fasciis*, and Oribasius' *De laqueis* and *De machinamentis*, commented and illustrated by Vidus in collaboration with the Florentine painter Francesco Salviati (9) (fig. 4).

If, in the study of the six Hippocratic works, Vidus had only a theoretical con-

tribution, the work of Galen *De fasciis* was richly illustrated with original designs of head bandages such as a complete capeline, single or combined with a gonion/chin double support sling, other types of bandages for craniocervical injuries, upper limb and thorax bandages, seen in

the Tabula LXXII, showing a shoulder immobilization technique used until today, such as the Desault bandage. Of the thoracoabdominal bandages, we mention the contention system used in the diastasis recti, where the bandages are arranged alternately, in the direction of the fibers of the oblique external and internal muscles of the abdomen and united in the midline as a raphe, which doubles the white line

(fig. 3).

Of the periarticular bandages, the intercrossed pattern slings of the thumb, groin and talocrural bandages conclude the series of 138 drawings of the *De fasciis* chapter.

From the work of Oribasius (AD 320-403), Vidus selected, commented and supplemented the chapters on knots and orthopedic devices to reduce dislocations and fractures.

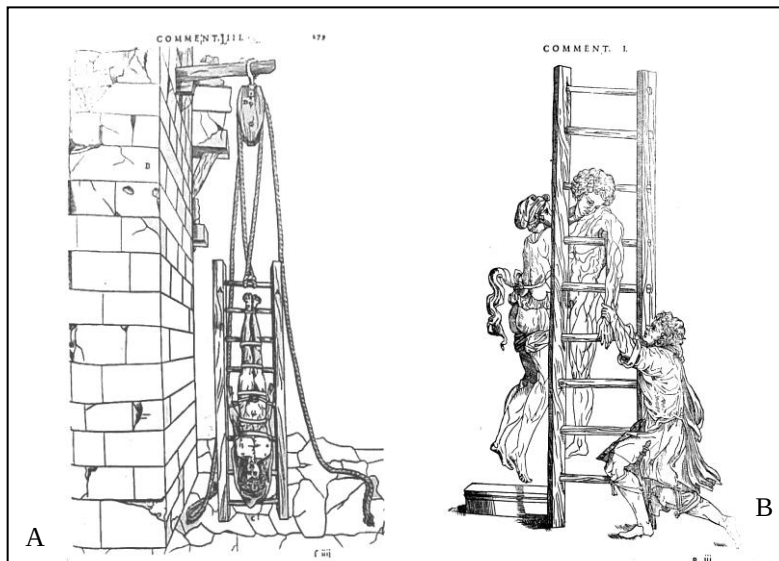


Fig. 4. Devices (machinamentes) for continuous extension (A) and luxation reduction (B) reproduced from original engravings of Francesco Salviati, preserved in Paris, at the Ecole Nationale Supérieure des Beaux-Arts, Inventario Masson, 210 (A) and Bibliothèque Nationale de France (B) Départements des Manuscrits (9).

In the work of Oribasius, the contribution of Vidus consisted in the diversification and improvement of the knot systems and especially of the outstanding graphical presentation that facilitated learning. Finally, the systems for the reduction of dislocations and fractures are initially presented segmentally, and then gradually assembled, indicating where to put the sprockets and pulleys thus making possible the replica-

tion of the device for any physician. The images of this last chapter of Guidi's *Chirurgia* were also made by Francesco Saviati by engravings on copper plates (fig. 4).

After the death of King Francis I (1547), Guido Guidi returned to Italy and settled in Pisa, where he practiced surgery and held public lectures of surgical anatomy through demonstrative dissections of human cadavers (10, 11, 12).

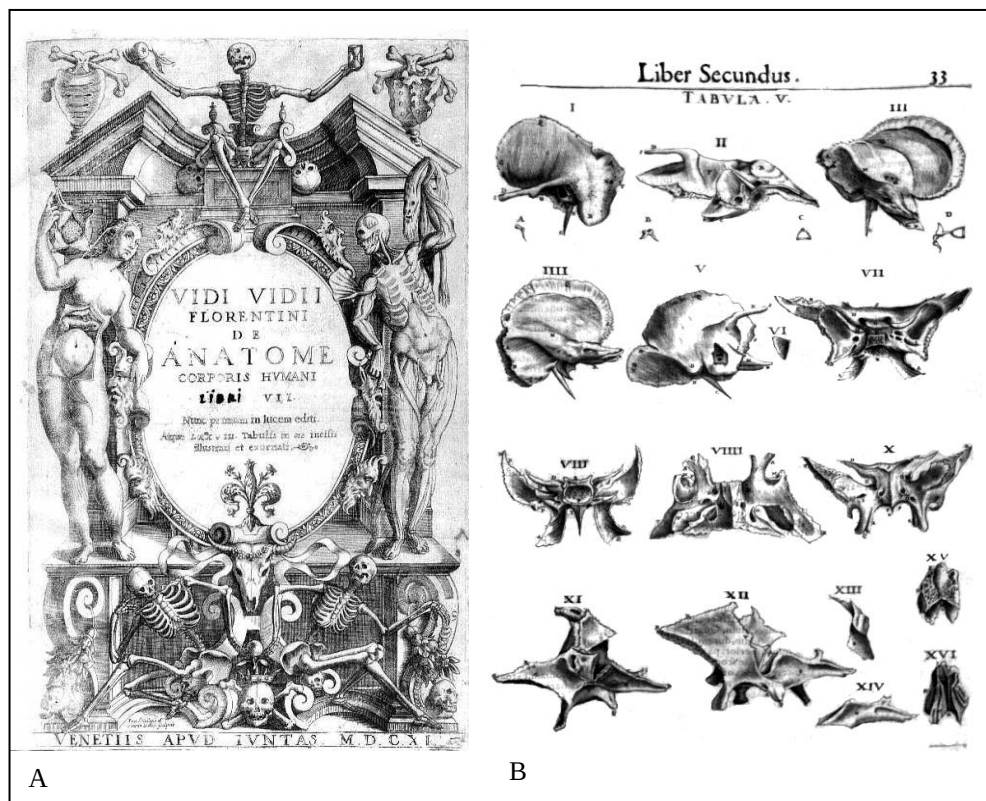


Fig. 5. A. The opening page of the Treaty *De Anatome*; B. Tabula V of Chapter II (Liber Secundus), which presents for the first time, the disarticulated bones of the skull base (7).

This allowed him to accumulate a notable experience in surgery and anatomy that enabled him to add new discoveries to the scientific heritage of the time. In the surgical field he was the first to describe the arterial aneurysms, an original technique of tracheotomy, a procedure of lengthening the foreskin of the penis incurvatus or other sequels of circumcision, cutaneous plasty of trophic limb ulcers, nose plasty, plaster or wax dental implants to improve phonation and he established the axillary lines as landmarks for the thoraco-brachial approach (13, 14).

He named in Italian language *vermiforme*, the ileocecal appendix, discovered by Berengario da Carpi (1460-1530) and

described the *ostia* of the hepatic veins openings in the inferior vena cava. In the accompanying drawing it can be observed a rare anatomical variation of five such *ostia*, demonstrating that the study was done by dissection on human corpses.

In the medical field, he was the discoverer of chickenpox. By studying diabetes, he concluded that it was a consequence of a single glandular insufficiency (without specifying the gland). He successfully applied the physiotherapy, using the therapeutic qualities of the baths of Pisa (15).

In the anatomy field, he wrote a remarkable treatise – *De anatome* (fig. 5) – in which he established the conditions of

building a *Theatrum Anatomicum* (5), presented, for the first time, the disarticulated bones of the skull base, described the orbital process of the palatine bone and the pterygoid canal that bears his name – *the vidian canal* (16).

CONCLUSIONS

Although forgotten today, Guido Guidi was a leading figure of the Renaissance medicine, both Italian and French (17, 18,

19). He was the first professor of anatomy and surgery at the Collège de France, and his *Chirurgia*, remained the reference book for teaching surgery in the following two centuries. It is considered the first original surgery book, beautifully illustrated by a famous contemporary Florentine painter. In the field of anatomy Guido Guidi was a prominent personality with important contributions that lasted until today as eponyms in *Nomina Anatomica*.

REFERENCES

1. König E., The great painters of the Italian Renaissance, H. F. Ullmann, 2007.
2. Grmeck, M.D., Contribution à la biographie de Vidius (Guido Guidi), premier lecteur royal de médecine: ses origines et sa vie avant la période parisienne, *Revue d'histoire des sciences*, 1978, 31, 4: 289-299.
3. Choulant L, *History and Bibliography of Anatomic Illustration*, Maurizio Martino Ed., Cambridge, 1945, p. 21-A-21-R.
4. Sterpellone L, *I Protagonisti della Medicina*, Antonio Delfino Ed., Roma, 2000, p. 108.
5. Brockbank W, *The man who was Vidius*, Ann Roy Coll Surg Engl 1956, 19: 269-295.
6. Choulant L, *History and Bibliography of Anatomic Illustration in its Relation to Anatomic Science and the Graphic Arts*. Translated and Edited by Frank M. The University of Chicago Press, Chicago, 1920.
7. Vidus Vidius, *Chirurgia è Graeco in Latinum conversa*, Vido Vidio Fiorentino interprete, cum nonnullis eiusdem Vidii comentariis, Bruxelles: Ed. Culture & Civilisation, in folio facsimile, 1970.
8. Cellini B., *La vita di Benvenuto Cellini scritta da lui medesimo*, Felice Le Monier editore, Firenze 1924.
9. Goguel C M., Francesco Salviati (1510-1563) o la Bella Maniera, *Electa*, 1998, pp. 131-135.
10. Hirsch A., *Biographisches Lexikon der hervorragenden Aerzte aller Zeiten and Völker*, Martino Publishing 2002, facsimile of 1885 ed., vol II, pp. 693-694.
11. Albert S. Lyons und R. Joseph Petrucelli II, *Die Geschichte der Medizin im Spiegel der Kunst*, Dumont, 2003, pp. 408-409.
12. Antonio Delfino Ed., *Storia della Medicina*, Seconda edizione, Roma, 1994, vol.2, pp. 339-412.
13. Regal W, Nanut M. *Ominöser Darmzipfel (Narrenturm 141)* Ärzte Woche, 22. Jahrgang Nr. 18, 2008.
14. Shane Tubbs R., Salter E G, *Vidus Vidius (Guido Guidi) (C.1509–1569)*, *Neurosurgery*, vol. 59 (1), July 2006, pp. 201–203.
15. Cocchi A, *Dei bagni di Pisa*, ed. Società Tipogr. de' classici italiani, Milano, 1842.
16. Vidus Vidius, *De anatome*, Veneția, 1560.
17. Dobson J, *Anatomical eponyms*, Livigstone ltd, 1962, pp. 83-85.
18. Forbis P., Bartolucci S.L., *Stedman's Medical Eponyms*, Williams & Wilkins, 1998.
19. Haller, von A., *Bibliotheca Anatomica*, 1774 facsimile, vol. I, pp. 236-237, Georg Olms Verlag Hildesheim-New York, 1969.
20. http://www.allposters.com/-sp/Bust-of-Cosimo-I-Posters_i12189498_.htm.

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B. Books

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These fees may be paid by bank transfer in the account of the Iasi Society of Physicians and Naturalists (**RO72RNCB017503360434001**).

INSTRUCȚIUNI PENTRU AUTORI

Revista Medico-Chirurgicală a Societății de Medici și Naturaliști din Iași publică, articole originale (*de cercetare fundamentală și clinică*), articole tip trecere în revistă (*review*) și actualități (*update*), articole tip prezentare de caz, note clinice și experimentale, recenzii, articole tip scrisoare către editor, informări asupra unor reuniuni științifice etc. în domeniul medicinei și în domeniile conexe. Nu sunt acceptate materiale deja publicate, în curs de publicare sau aflate în evaluare la alte reviste, cu excepția lucrărilor publicate în rezumat la reuniuni științifice.

Revista apare în limba engleză (*on-line* și tipărit) cu o versiune *on-line* în limba română justificată de necesitatea prezervării și dezvoltării lexicului medical românesc precum și pentru asigurarea răspândirii rapide a informației științifice în mediul medical din România. Articolele care cuprind cel puțin un autor din România vor trimite ambele versiuni (versiunea *on-line* în limba română va avea obligatoriu diacritice și va folosi ortografia cu *ă* și *sunt*). Evaluarea calității articolului se va realiza pe versiunea în limba engleză.

Pentru **articolele originale** conținutul trebuie structurat în următoarele secțiuni: INTRODUCERE, MATERIAL ȘI METODE, REZULTATE, DISCUȚII, CONCLUZII, BIBLIOGRAFIE. Manuscrisele vor respecta cerințele generale impuse de Comitetul internațional al editorilor jurnalelor medicale (*International Committee of Medical Journals Editors-ICMJE*) disponibil la adresa <http://www.icmje.org/icmje-recommendations>.

Pentru articolele tip **prezentare de caz**, conținutul trebuie structurat în următoarele secțiuni: INTRODUCERE, PREZENTAREA CAZULUI, DISCUȚII, CONCLUZII, BIBLIOGRAFIE.

Articolele vor fi depuse împreună cu o scrisoare de însoțire care va atesta ca:

- manuscrisul a fost aprobat de toți autorii;
- a fost obținută permisiunea de publicare de la instituția în care a fost efectuată lucrarea;
- articolul nu a fost publicat și nu este în curs de evaluare sau publicare în nici o formă, la o altă revistă indiferent de tipul acesteia;
- autorii acceptă transferul necondiționat și complet al dreptului de autor la *Revista Medico-Chirurgicală a Societății de Medici și Naturaliști din Iași*;
- autorii își asumă în întregime responsabilitatea în ceea ce privește comportamentul științific și regulile etice (inclusiv plagiatul);
- autorii precizează orice conflict de interese cu privire la manuscris
- contribuția fiecărui autor este menționată în cadrul articolului;
- au fost respectate prevederile referitoare la drepturile de participare ale oamenilor și animalelor în cercetare (detalii la www.revmedchir.ro).

PREGĂTIREA MANUSCRISULUI

Pregătirea manuscrisului se va face conform instrucțiunilor publicate în: Uniform requirements for manuscripts submitted to biomedical journals (1994) *Lancet* 1996; 348; V2; 1-4.

Textul va fi scris cu fonturi Times New Roman 12 pts. la două rânduri, rândurile fiind numerotate. Numerotarea paginilor se face în partea din dreapta jos.

Abrevierile nu trebuie utilizate excesiv și trebuie explicate la prima apariție în text.

Materialele nu vor depăși: articole originale - 6 pagini/2.500 cuvinte; articole tip trecere în revistă (*review*) și actualități (*update*) - 8 pagini/3500 cuvinte; articole tip prezentare de caz - 4 pagini/1.700 cuvinte; note clinice și experimentale - 3 pagini/1.300 cuvinte; recenzii, articole tip scrisoare către editor, informări asupra unor reuniuni științifice - o pagină/450 cuvinte. Revista își rezervă dreptul unor modificări impuse de tehnoredactare și aranjare în pagină.

Aceleași reguli se aplică versiunii în limba română.

Manuscrisele respinse nu se returnează.

Titlul și autorii

Titlul trebuie să fie cât mai scurt, elocvent pentru conținutul manuscrisului. Numele autorilor vor fi scrise cu litere de rând, prenumele precedând numele, pentru autori prescurtat, pentru autoare întreg. Afilierea va cuprinde instituția (Universitate și Facultate/Spital) și departamentul pentru fiecare autor. Autorii sunt obligați să menționeze **după bibliografie** autorul desemnat pentru corespondență, cu datele de contact (e-mail și telefon).

Rezumatul (cercetări originale)

Componente obligatorii	Recomandări speciale
Obiectivele clare ale studiului	Extrageți elementele principale din fiecare etapă a studiului pentru a fi expuse cât mai concentrat
Descrierea materialului și metodelor utilizate (concis și clar)	În rezumat NU vor fi incluse:
Rezultatele obținute (cele mai importante, exprimate, dacă este posibil, numeric și evaluate statistic)	* tabele, figuri sau note bibliografice
Concluziile cele mai importante și semnificația lor științifică	* informații care nu sunt prezentate în studiu
	* mai mult de 250 de cuvinte

Întrucât lucrările sunt publicate în limba engleză, redactarea lor trebuie să fie conform normelor internaționale. Rezumatul va fi precedat de titlul articolului, scris cu majuscule, urmat de cuvântul (Abstract), conform următorului model:

NEUROLOGICAL MANIFESTATIONS IN COLAGEN DISEASES (Abstract): Collagen disease are ... (urmează textul scris în continuare, fără aliniate). La sfârșitul rezumatului se vor menționa 3-5 cuvinte cheie scrise cu majuscule.

Subtitlurile din cuprinsul articolului trebuie scrise cu litere capitale.

Instrucțiuni pentru autori

Figurile și tabelele trebuie inserate echilibrat în text. Fiecare figură sau tabel trebuie să fie însoțite de titlu și legendă.

Figurile, numerotate cu cifre arabe, vor avea o lățime de 6,5 cm (pentru a se încadra pe o coloană) sau de 13,5 cm (pentru a se încadra pe ambele coloane) și vor fi menționate în text în paranteză cu caractere drepte (fig. 1). Figurile trebuie să îndeplinească următoarele condiții:

- fotografiile color scanate la o rezoluție de minim 300 dpi și prelucrate ulterior pe calculator, fișier original (*.TIFF, *.JPG);

- imaginile color (desene, grafice) realizate pe calculator (în alb-negru cu contrast), cu text lizibil, menționate în text, fișier original (*.XLS, *.CDR); prelucrarea imaginilor trebuie redusă la minimum, fiind acceptate doar scalări și/sau eventual modificări ale contrastului și luminozității; adăugarea de indicatori/marcaje care să permită identificarea anumitor puncte de interes este permisă; acestea vor fi însoțite de o legendă adecvată; modificările imaginilor susceptibile a altera semnificația acestor nu sunt acceptate.

Titlul și legenda figurilor vor fi scrise conform modelului:

Fig. 2. Rezultatele clonării moleculare

Tabelele (editate cu font Arial Narrow) vor fi numerotate cu cifre romane, menționate în text în paranteze (tab. III), vor fi inserate în text și vor avea o lățime de 6,5 cm sau de 13,5 cm, fără să depășească lățimea unei pagini. Titlul tabelului, scris cu majuscule, formatat stânga-dreapta va fi poziționat deasupra tabelului conform modelului:

TABELUL III

Date analitice comparative

Bibliografia (cu majuscule)

Referințele bibliografice trebuie numerotate cu cifre arabe *în ordinea apariției în text* (deci nu în ordine alfabetică). Bibliografia, citată conform modelului Vancouver, va fi plasată la sfârșitul articolului și va cuprinde numai referințele citate în text (prin menționarea în paranteză a numărului referinței). Abrevierea titlurilor revistelor se va face conform *Index Medicus*.

Dacă un articol are maximum 6 autori vor fi transcrise toate numele. În cazul în care sunt 7 sau mai mulți autori, se vor menționa doar numele primilor 3 autori la care se adaugă abrevierea et al.

Sunt acceptate următoarele forme de scriere a bibliografiei:

A. Articole din reviste:

Amura SG. Neurotransmitter transportor: recent progress. *Ann Rev Neurosci* 1993; 16: 73-93.

Tonis PJ, von Sandick JW, Nieweg OE et al. The hidden sentinel node in breast cancer. *Eur J Nucl Med* 2002; 29: 305-311.

B. Cărți:

- *Titlul unei cărți:*

Ashcroft FM. *Ion Channels and Disease*. San Diego, London: Academic Press, 2000.

- *Capitol dintr-o carte:*

Pandiom MR, Fischer DA. Immunoradiometric assays. In: Scabes CG editor. *Handbook of Endocrine Research Techniques*. New York, London: Academic Press, 1993, 26-52.

TRIMITEREA MANUSCRISULUI

Manuscrisele, după înregistrare la Secretariatul S.M.N., vor fi încărcate în format electronic pe platforma <http://pkp.umfiasi.ro/revmedchir/index.php/RMC> (se acceptă doar fișiere .docx). Durata minimă de la depunere la publicare este de 90 zile (timp necesar pentru procesare și peer-reviewing). La publicare, redacția va menționa data primirii articolului.

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Acceptarea articolului pentru publicare implică asumarea transferului dreptului de proprietate intelectuală al autorilor asupra revistei.

Formularul pentru *copyright* va fi semnat de autorul desemnat pentru corespondență.

În atenția autorilor

Deoarece este necesară contribuția materială a autorilor la cheltuielile de editare, tipărire și webdesign ale *Revistei Medico-Chirurgicale* se impun următoarele condiții:

1. Membrii cotizanți ai *Societății de Medici și Naturaliști Iași* pot publica articole fără achitarea unei taxe.

2. Autorii care nu sunt membri cotizanți ai *Societății de Medici și Naturaliști Iași* vor achita o taxă de 100 RON/articol în contul **RO72RNCB0175033604340001**, chitanța fiind prezentată odată cu depunerea articolului.

3. Începând cu data de 1 ianuarie 2015, pe site-ul www.revmedchir.ro vor fi accesibile gratuit numai rezumatele articolele publicate. Accesul online la formatul integral al articolelor va fi asigurat numai după achitarea următoarelor taxe:

- 10 €/articol, format .pdf sau .jpeg, trimis solicitantului pe adresa sa de e-mail; pentru solicitarea a 10 sau mai multe articole de către aceeași persoană taxa este de 7 €/articol.

- 300 €/an acces nelimitat *on-line* la toate articolele publicate în revistă.

Aceste taxe se pot achita și prin transfer bancar în contul *Societății de Medici și Naturaliști Iași* (**RO72RNCB017503360434001**).