

LETTER TO THE EDITOR

TREATMENT WITH A COMBINATION OF OMALIZUMAB AND SPECIFIC IMMUNOTHERAPY FOR SEVERE ANAPHYLAXIS AFTER A WASP STING

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Hymenoptera venom anaphylaxis after bee or wasp sting is a common problem that affects about 1.2% to 3.5% of the general population. Venom-specific immunotherapy (VIT) is an established mode of treatment for immunoglobulin (Ig) E-mediated Hymenoptera venom allergy. However, VIT may often be associated with immediate anaphylaxis which can lead to treatment withdrawal. Several cases published in recent years suggest that omalizumab, used as add-on therapy may be able to prevent anaphylaxis during VIT. We report the case of a 30-year-old woman, suffering from mild persistent asthma, who had a history of severe anaphylactic reactions after yellow jacket sting, and after eating peanuts, contact with guinea pig hair, and i.v. administration of dexamethasone sodium phosphate. Initial specific immunotherapy had to be stopped due to severe anaphylaxis (hypotension, dyspnea, and angioedema). The immunotherapy was reintroduced accompanied by the anti-immunoglobulin (Ig) E monoclonal antibody omalizumab. Subcutaneous omalizumab 150 mg was initiated 4 weeks after the anaphylaxis incident and 1 day before the resumption of VIT. Rush treatment was uneventful, and the usual cumulative dose of 111.1 µg was successfully reached. The combination of omalizumab and VIT is a valid option of therapy for these patients and could reduce asthma and food allergy symptoms.

Hymenoptera venoms are the source of many allergens that could lead to large local reactions (LLRs) and systematic reactions (SRs). In Europe the most frequent and clinically important are stings from the *Apoidea* (*Apis mellifera*, *Bombus* spp.) and *Vespidae* (*Vespinae* and *Polistinae* subfamilies). Epidemiological studies showed a prevalence of 0.3-8.9% for SRs, and 0.3-42.8% for general anaphylaxis (1, 2).

Venom immunotherapy (VIT) is effective in preventing anaphylactic sting reactions. It has been established that the efficiency of wasp VIT is 95% and 75-85% for bee-venom-allergic patients. However, such therapy is associated with a risk of treatment

associated with allergic, mainly anaphylactic, side effects occurring in up to 20% of all cases (3).

In recent years, omalizumab, a recombinant humanized monoclonal antibody has been used in premedication of VIT and it has been shown to successfully reduce the incidence of systemic allergic reactions (SAR) which can occur upon VIT (4).

In this report we describe a patient's successful treatment with omalizumab and VIT after a severe anaphylactic reaction caused by the sting of a yellow jacket.

Case description

A 30-year-old atopic woman who experienced a

Key words: venom-specific immunotherapy, anaphylaxis, omalizumab

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Table I. Specific IgE (IgEs) using a microarray chip (ImmunoCAP® ISAC).

Allergens	IgEs level (ISU-E)
Grass	
nCyn d	36
rPhl p 1	77
rPhl p 2	40
rPhl p 5	63
rPhl p 6	7.1
rPhl p 11	25
Tree	
rBet v 1 (PR-10 protein)	80
nCry j 1 (Pectatelyase)	1.7
nCup a 1 (Pectatelyase)	3.4
nOle e 1 (Common olive group 5)	0.3
nPla a 2 (Polygalacturonase)	2.4
Animals	
rFel d 1 (Ureteroglobulin)	15
rCan f 1 (Lipocalin)	3.4
nMus m 1 (Lipocalin)	1.7
rEqu c 1 (Lipocalin)	0.5
Alternaria	
rAlt a 1 (Acidic glycoprotein)	2.5
Hymenoptera	
rVes v 5 (Antigen 5)	2.9
rPol d 5 (Antigen 5)	1.3

IV grade anaphylactic reaction to a wasp sting, according to Mueller's classification (5), was admitted to hospital in order to carry out VIT. Since childhood she had suffered from asthma and allergic rhino-conjunctivitis. The subject experienced severe anaphylactic reactions after eating peanuts, contact with guinea pig hair and i.v. administration of dexamethasone sodium phosphate.

Intradermal skin tests were positive to yellow jacket venom at 0.01 µg/ml and negative to bee venom. Serum specific IgE to wasp venom was 2.62 kU/L, and total IgE was 1239.36 kU/L. Specific IgE against other allergens were performed by ImmunoCAP and ISAC (Table I). The tryptase level was normal (1.83 µg/L). The physical examination, chest X-ray and ECG were without abnormalities. The spirometry and laboratory tests were also normal.

VIT was highly advisable (6). The first inpatient VIT rush was initiated with antihistamine pretreatment (Clemastine fumarate 1 mg orally, 12 and 2 hours before), but this was stopped after 8 µg of Venomenhal® wasp due to anaphylaxis (hypotension, dyspnea, and angioedema). After four weeks, one day before VIT, the subject received a subcutaneous injection of 150 mg of omalizumab (Xolair®) (Table II). The following monthly doses were well tolerated. Omalizumab pretreatment was continued monthly 1 day before VIT.

DISCUSSION

It is established that VIT is a highly effective treatment which reduces the risk of serious anaphylactic reactions, morbidity and mortality, and improves quality of life. However, the major problem of VIT is severe anaphylactic reaction during the initial phase of this treatment (7). A European multicentre study reported a greater risk of SRs during the incremental phase of VIT in female patients receiving beta-blockers, those suffering from mastocytosis and those treated with honeybee venom (8, 9). Our patient did not take beta-blockers and mastocytosis was excluded but her medical history indicated strong sensitization to numerous allergens. ImmunoCAP analysis provided evidence of elevated concentrations of serum specific IgE against different allergens (Table I). Total serum IgE was also very high. Galli and Tsai (10) found

Table II. Protocol for administration of wasp venom immunotherapy (VENOMENHAL® Wasp).

Time	Treatment
Day 1	beginning of treatment with Venomenhal® subcutaneous injection of Venomenhal® 0.001, after 30 min 0.01, after 30 min 0.1, after 30 min 1.0, after 30 min 2.0, after 30 min 4.0, after 30 min 8.0 mcg, stopped after 8.0 mcg of venom due to anaphylaxis.
One month later	a new modified VIT was administered
Week 4	1 subcutaneous injection of omalizumab 150 mg, Clemastinum 2 mg i.v. and after one day Venomenhal 8 mcg (well tolerated).
Week 8	1 subcutaneous injection of omalizumab 150 mg, Clemastinum 2 mg i.v. and after one day Venomenhal 16 mcg (well tolerated).
Week 16	1 subcutaneous injection of omalizumab 150 mg, Clemastinum 2 mg i.v. and after one day Venomenhal 20 mcg (well tolerated).
Week 20	1 subcutaneous injection of omalizumab 150 mg, Clemastinum 2 mg i.v. and after one day Venomenhal 40 mcg (well tolerated).
Week 24	1 subcutaneous injection of omalizumab 150 mg, Clemastinum 2 mg i.v. and after one day Venomenhal 60 mcg (well tolerated).
Week 28	1 subcutaneous injection of omalizumab 150 mg, Clemastinum 2 mg i.v. and after one day Venomenhal 80 mcg (well tolerated).
Week 32	1 subcutaneous injection of omalizumab 150 mcg, Clemastinum 2 mg i.v. and after one day Venomenhal 100 mcg (well tolerated).
Future therapy:	
Every month	1 subcutaneous injection of omalizumab 150 mg, Clemastinum 2 mg i.v. and after one day Venomenhal 100 mcg.

that an increased level of IgE is convincingly linked to the pathophysiology of anaphylaxis and other acute allergic reactions. Pretreatment with an H1

antihistamine has been demonstrated to reduce the side effects of VIT. The patient was prescribed antihistamine premedications before VIT, but this

did not prevent anaphylaxis at the dose of 8 µg of venom.

Considering the high risk to the patient, we looked for an alternative pretreatment. There are several case reports on patients allergic to bee venom which describe the ability to tolerate VIT after pretreatment with omalizumab (11). Despite the patient's allergic history and poorly controlled asthma, omalizumab (150 mg) was administered subcutaneously one day before VIT (Table II). Omalizumab is a humanized mAb currently used mainly for treatment of moderate (US label) and severe asthma (US and EU labels). The mechanism by which omalizumab is responsible for the improvement of the course of allergic asthma is by binding to the C3 domain of the heavy chain of IgE. In this way omalizumab reduces up to 98% of serum IgE. Another effect of this medicine is down regulation of FcεRI on mast cells, basophils and dendritic cells (12, 13). Omalizumab has been shown to decrease the early and late phase anaphylactic reaction (14). The mechanism by which omalizumab might be working in this case is probably by decreasing the IgE level and allergic inflammation. Nevertheless, to date the optimal time and appropriate dosage for omalizumab administration during VIT to be used still remain unknown.

The combination of omalizumab and VIT may be a good option for patients who do not tolerate VIT, and are at high risk of anaphylaxis. During clinical observation, the course of asthma and the quality of life of the patient improved.

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LETTER TO THE EDITOR

MANAGEMENT OF IMMUNOSUPPRESSION AND ANTIVIRAL TREATMENT BEFORE AND AFTER HEART TRANSPLANT FOR HIV-ASSOCIATED DILATED CARDIOMYOPATHY

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Infection with HIV may lead to the development of cardiomyopathy as improved antiretroviral regimens continue to prolong patient life. However, advanced therapeutic options, such as heart transplant, have until recently been precluded to HIV-positive persons. A favorable long-term outcome has been obtained after kidney or liver transplant in HIV-positive recipients fulfilling strict virological and clinical criteria. We recently reported the first heart transplant in a HIV-infected patient carried out in our center. In this article, we detail the major challenges we faced with the management of antiretroviral and immunosuppressive treatments over the first 3 years post-transplant. The patient had developed dilated cardiomyopathy while on antiretroviral treatment with zidovudine, lamivudine and efavirenz. He was in WHO Stage 1 of HIV infection and had normal CD4+ count and persistently undetectable HIV-RNA. In spite of cardiac resynchronization therapy and maximal drug therapy, the patient progressed to end stage heart failure, requiring heart transplant. He was placed on a standard immune suppressive protocol including cyclosporine A and everolimus. Despite its potential pharmacokinetic interaction with efavirenz, everolimus was chosen to reduce the long-term risk of opportunistic neoplasia. Plasma levels of both drugs were monitored and remained within the target range, although high doses of everolimus were needed. There were no infectious, neoplastic or metabolic complications during a 3-year follow-up. In summary, our experience supports previous data showing that cardiac transplantation should not be denied to carefully selected HIV patients. Careful management of drug interactions and adverse events is mandatory.

Infection with the human immunodeficiency virus (HIV) may affect virtually every organ system and requires a life-long antiviral combination treatment that carries a high rate of adverse events (1).

The circulatory system may be affected in a number of ways by HIV infection and/or

antiretroviral treatment (ART) (2). The most common cardiovascular disorders observed in the HIV setting include coronary artery disease (CAD), pulmonary arterial hypertension (PAH) and idiopathic or non-ischemic cardiomyopathy (3, 4). This translates into a significant cardiovascular morbidity and mortality

Key words: pharmacokinetics, immunosuppression, immunodeficiency, antiviral agents

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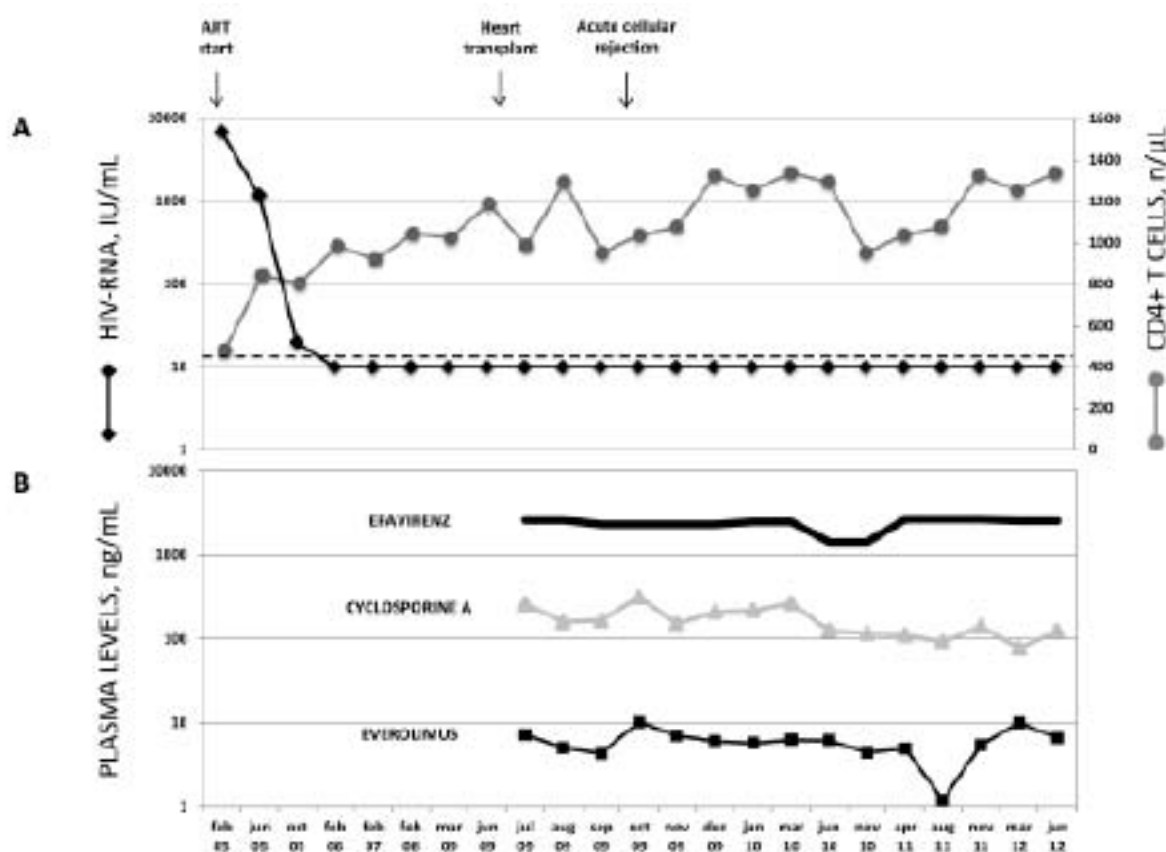


Fig. 1. Dynamics of HIV-RNA, CD4+ cell counts, antiretrovirals and immunosuppressors in the index case. Panel A) Kinetics of HIV-RNA and CD4+ T lymphocytes since diagnosis of infection throughout the post-transplant course. Panel B) Plasma level measurements of efavirenz, cyclosporine A and everolimus over time after heart transplant. HIV-RNA was measured in IU/mL; CD4+ lymphocytes were measured as cells/mL; cyclosporine A, everolimus and efavirenz plasma levels were measured in ng/mL. ART: antiretroviral treatment; dotted line represents the HIV-RNA limit of detection.

in HIV-positive individuals. Moreover, as improved ART efficacy continues to prolong life of HIV-infected persons, end stage organ failure, including heart failure, is increasingly recognized.

Despite this, advanced therapeutic options for heart failure, such as heart transplant, have until recently been precluded to HIV-positive persons. This is due to several reasons: i) HIV infection has traditionally been viewed as a deadly disease with a poor long-term prognosis; ii) concern exists that the intensive immune suppression that follows heart transplant may enhance the HIV-related risk of opportunistic disorders; iii) major interactions may occur between ART and immune suppressants (5).

In the face of these concerns, accumulating evidence suggests that a favorable long-term outcome may be obtained after kidney or liver transplant

in HIV-positive recipients (6). This has prompted individual US heart transplant centers to wait list and transplant HIV-infected patients fulfilling strict virological and clinical criteria (7-9).

We recently reported the first heart transplant in an HIV-infected patient carried out in our center (10). In the present article, we describe in detail the major challenges we faced in this case with the management of antiretroviral and immunosuppressive treatments over the first 3 years post-transplant, highlighting the issues related to wait list decision making, complication surveillance and management of the complex pharmacologic regimen.

Clinical case description

A 36-year-old male was admitted to our hospital in May 2009 because of severe heart failure that

Table I. Pre-transplant hemodynamic parameters of the case patient as measured during right heart catheterization at baseline and after vasodilation induction via administration of 100% oxygen plus epoprostenol infusion.

Parameter	Baseline	Induction
Central venous O ₂ saturation, %	59	72
Arterial pressure, mmHg	105/70	95/50
Pulmonary artery trunk pressure, mmHg		
Systolic	60	50
Diastolic	38	27
Mean	50	45
Mean pulmonary artery wedged pressure, mmHg	30	30
Total pulmonary vascular resistances, (Wood units/m ²)	25	15
Arteriolar pulmonary vascular resistances (Wood units/m ²)	10	5
Post-capillary pulmonary vascular resistances (Wood units/m ²)	15	10
Cardiac Index (liters/min/m ²)	2	3

Table II. Current requirements of the Italian National Transplant Center for wait listing of potential HIV-positive kidney and liver recipients.

Criteria	Fulfillment by the index case
Ability to provide informed consent	✓
ART naïve patient with CD4+ cell count persistently above 200/μl	✗
Patient on ART with CD4+ cell count stable since 12 months and HIV-RNA undetectable at the time of listing	✓
Compliance with ART and prophylaxis of opportunistic infections	✓
Willingness to stay on ART and prophylaxis	✓
Negative pregnancy test	NA
Absence of advanced liver disease due to chronic hepatitis B, D and C virus infection (except for liver transplant candidates)	✓
Ability to perform 3-monthly screening of CD4+ cell count and HIV-RNA load in the transplant center	✓

NA: not applicable

was refractory to medical treatment, including intravenous infusion of diuretics, vasodilators and inotropes. He had no family history of sudden death, cardiomyopathy or muscle disorders and there were no relatives with known CAD.

In January 2005, he had been diagnosed with HIV infection following an at-risk sexual conduct that drove him to undergo the test. At the initial evaluation, his HIV-RNA load was 6900 IU/ml (Cobas Amplicor HIV-1 Monitor, Roche, Basel, Switzerland) and his CD4+ T lymphocyte count was 480 cells/ml, with a CD4+ to CD8+ ratio of 0.8. He was negative for hepatitis B virus surface antigen and hepatitis C virus RNA. There were no other signs of disease. A 12-lead ECG was normal and the chest x-ray showed a normal cardiac silhouette. The patient was fit and had no shortness of breath, chest pain or palpitations on exertion. According to the clinical staging of HIV infection (Centers for Disease Control Classification Category A2 / World Health Organization Stage 1), the patient was started on ART with zidovudine (AZT, 300 mg bid) and lamivudine (3TC, 150 mg bid) in a single tablet combination formulation plus efavirenz (EFV, 600 mg daily). On this combination ART, he obtained a rapid and persistent clearance of HIV-RNA from 4 months of therapy and a fast and durable restoration of the CD4+ cell count. Fig. 1 presents the kinetics of HIV-RNA and CD4+ cells observed in this patient over time. The patient did not subsequently experience any metabolic derangement or impairment of liver or kidney function. No viral breakthrough occurred.

Two years after starting ART, the patient began to complain of shortness of breath on mild exertion and progressive orthopnea. In April 2007, he experienced recurrent paroxysmal nocturnal dyspnea, that led him to undergo an echocardiographic study. The ultrasound pattern was consistent with overt dilated cardiomyopathy. The patient was then referred to our hospital for further evaluation, and was put on lisinopril, furosemide, potassium canrenoate, digoxin and aspirin. In July 2007, a coronary angiogram showed normal epicardial coronary arteries. An overnight blood oxygen monitoring ruled out a sleep-disordered breathing. Carvedilol was added to the medical treatment. Subsequently, the patient's clinical conditions improved while he was on the same three-drug ART.

In June 2008, a 24-hour electrocardiographic monitoring displayed >2.5 second pauses, a left bundle branch block and several triplets of ventricular ectopic beats. An echocardiogram showed a left ventricular diastolic dimension of 7.3 cm, a 33% ejection fraction, a restrictive mitral pattern with severe regurgitation and a dilated left atrium. In March 2009, Doppler tissue imaging revealed significant intra- and inter-ventricular delays, leading to the implant of a bi-ventricular automatic cardioverter defibrillator. At this time, the echocardiogram showed worsening of cardiac structural and functional parameters, with dilation of the right ventricle, a tricuspid annular plane systolic excursion of 12 mm and a systolic pulmonary artery pressure of 50 mmHg.

Despite cardiac resynchronization therapy and maximal drug therapy, the patient experienced recurrent episodes of decompensation. In June 2009, he was admitted again to our hospital with end stage heart failure. The results of the right heart catheterization are shown in Table I.

A multidisciplinary assessment was therefore carried out. A destination artificial heart was deemed unethical due to the patient's age and absence of significant pulmonary, renal or hepatic disorders as well as overt neoplasia. In contrast, as shown in Table II, the patient appeared to fulfill all existing requirements of the Italian National Transplant Center for wait listing of potential HIV-positive kidney and liver recipients. Indeed, his HIV-RNA had been undetectable during the preceding 4 years, his CD4+ cells were normal (Fig. 1) and he had not experienced any AIDS-defining illness or opportunistic infection for more than 3 years. A decision was therefore made to list the patient for heart transplant with an emergency criterion and transfer the patient to the cardiac surgical intensive care unit. Pre-transplant virological assessment showed undetectable HIV-RNA, positive anti-Epstein Barr virus (EBV) and Cytomegalovirus (CMV) immunoglobulin G and negative Venereal Disease Research Laboratory and Treponema Pallidum Hemagglutination tests.

Twenty-four hours later, the patient received an AB0 compatible, weight-matched orthotopic heart transplant. The donor was an HIV-negative 42-year-old female, with blood group B Rh-factor positive, who had died due to severe anoxic brain damage

ensued after a caesarean section. A standard immune suppressive protocol was initiated. Priming was carried out with anti-thymocyte globulin, starting before graft reperfusion, at the dose of 1.5 mg/kg/day as intravenous infusion for 5 days coupled with 125 milligrams twice daily boluses of metil-prednisolone for 3 days. From the fourth post-transplant day, metilprednisolone was tapered off from 125 mg bid iv to 10 mg bid po and cyclosporine and everolimus were added. Everolimus was chosen with the aim to reduce the long-term risk of opportunistic neoplasia. The initial oral doses were 250 mg bid for cyclosporine and 0.75 mg bid for everolimus. The target plasma trough levels were 250-300 ng/ml for cyclosporine and 3-8 ng/ml for everolimus. Due to its enduring efficacy, ART was continued as previously without any changes, despite the known interference between EFV and everolimus, and prophylaxis with co-trimoxazole was added to the oral regimen.

As shown in Fig. 1, EFV plasma concentration, measured between July 2009 and April 2011 by a validated high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method (limit of quantification 1.000-4.000 ng/ml), remained in the target therapeutic range. Everolimus daily dose was progressively increased over the first few weeks post-transplant, up to a dose of 3.75 mg/day, to keep plasma levels above 3 ng/ml.

The post-transplant course was uneventful and there was no right ventricular dysfunction. The patient was discharged on the 12th post-transplant day after a normal endomyocardial biopsy result.

The patient was subsequently followed up every week with clinical exam, endomyocardial biopsy, echocardiogram and chest x-ray. At each visit, blood tests were performed, including measurement of cyclosporine and everolimus plasma levels, CD4+ and CD8+ cell counts, HIV-RNA and CMV-DNA quantitation and complete metabolic screening. In the fourth post-transplant month, the patient experienced an International Society for Heart and Lung Transplant grade 2R acute cellular rejection, that was treated with three boluses (1 gram per day) of metil-prednisolone iv. No CMV or EBV replication was detected. There were no infectious or neoplastic complications during a 36-month follow-up. Serum levels of cholesterol and tryglicerides were kept within normal values through

administration of omega polienoic acids, 3 grams per day, and fluvastatin, 40 mg per day. The follow-up echocardiogram performed at month 36 post-transplant showed normal left ventricle structure and function (diastolic and systolic diameters of 43 and 26 mm, respectively; interventricular septum 12 mm; ejection fraction 58%). Right ventricle was also normal for size and radial contractility, with a slight reduction in tricuspid annular plane systolic excursion of 16 mm. At the same time, He scored 24 points on the Minnesota Living with Heart Failure Questionnaire, suggesting a mild impairment of his quality of life.

DISCUSSION

The enhanced efficacy of current regimens of highly active ART, coupled with improved protocols of immune suppression, have led individual heart transplant centers in the US to wait list and transplant carefully selected patients with known HIV infection. We recently reported the first successful heart transplant in an HIV-infected, dilated cardiomyopathy patient, performed in Europe (10). In this article, we describe in detail our experience with this case. The procedure was uneventful and our 36-year-old patient recovered fully within a few days. Of note, no opportunistic infection or neoplasia was observed during the first post-transplant year and there was no evidence of liver, kidney, metabolic or bone marrow dysfunction. However, we did experience therapeutic challenges in the management of this patient's medications. In particular, as everolimus is a substrate of the cytochrome P450 3A4 and the multidrug efflux pump P-glycoprotein, both potently induced by efavirenz, we attained everolimus plasma levels steadily within the recommended therapeutic range using doses of this drug three-times higher than usually used in our center. Just before transplant was performed, we decided not to change the antiviral regimen including AZT, 3TC and EFV. This decision was based on the high efficacy of this combination schedule as well as the absence of related adverse events during the prior 4 years. As far as immunosuppression was concerned, the decision to start everolimus in association with cyclosporine A, despite the anticipated interference, was multifactorial. Firstly, everolimus was shown

to favorably impact the incidence of allograft vasculopathy (11). Secondly, it would allow us to keep cyclosporine A plasma levels within a lower range, thereby reducing the chance of opportunistic infections (in particular CMV reactivation) and post-transplant renal dysfunction, considering the additive risk of HIV-induced nephropathy. Finally, everolimus was chosen because of its proven effect on the emergence of post-transplant lymphoproliferative disorders (12), also a common late complication of HIV infection (13).

Our experience may not be short of consequences as heart transplant becomes an increasingly employed option for HIV+ patients (14). A number of cardiac manifestations of HIV infection have been described, including pericarditis, endocarditis, myocarditis with or without cardiomyopathy, primary pulmonary hypertension and CAD (2). The prevalence of cardiac disease in HIV-infected individuals is uncertain as it depends on the population studied (patients naïve or on ART). HIV infection has been recognized, however, as a risk factor for non-ischemic dilated cardiomyopathy. Also, the mortality rate of HIV-related cardiomyopathy appears to be four times higher than that of uninfected subjects with idiopathic cardiomyopathy (2, 3).

Before the initiation of ART, left ventricular dysfunction associated with myocarditis, cardiotropic viral infection and, often, right ventricular involvement, was common in HIV-infected patients (15-17), suggesting a direct deleterious effect of viral infection on the heart. Subsequently, data have accumulated to suggest that ART may also play a role in the development of myocardial disease. For instance, AZT was shown to cause mitochondrial myopathy secondary to destruction of cardiac mitochondrial ultrastructures and inhibition of cardiac mitochondrial DNA replication (18, 19), although conflicting data have also been published (20). In fact, although ART use has been associated to an overall reduction of mortality due to congestive heart failure in HIV-positive patients, the actual effects of different antiviral regimens may significantly vary.

Pulmonary hypertension is also a commonly observed condition in HIV-infected patients and is generally unrelated to thromboembolic pulmonary disease, intravenous drug use, AIDS-defining

illnesses, pulmonary infections, low CD4+ cell counts or hypoxemia (21, 22).

As far as HIV and CAD are concerned, a large prospective study showed that HIV-positive patients are at a higher risk to develop a myocardial infarction, mostly as a function of the years of exposure to ART (23). In this context, a major role may be played by dyslipidemia, glucose intolerance and the metabolic dysfunction associated with ART (24, 25) as well as by traditional cardiovascular risk factors that are independent of HIV infection, such as cigarette smoking (26, 27).

In our patient, the etiology of the cardiomyopathy remains unclear. CAD was ruled out by a normal coronary angiogram, but we could not exclude neither an AZT-induced myocardial damage nor a direct effect of viral infection.

The results of our clinical experience support the conclusions of other heart transplant groups with larger experience in the HIV setting that cardiac transplantation should be contemplated among the therapeutic options in 'carefully selected' patients with HIV showing undetectable viral load, normal CD4+ cell counts, and no history of opportunistic infections (8, 9, 14, 15). This has been recently confirmed by the group of New York. In a retrospective study of 1,679 cardiac transplants, seven HIV-positive patients with mean CD4+ cell count of 554 ± 169 cells/ μ l and undetectable viral load at the time of transplantation were identified. In this subset of patients, the current follow-up suggests that survival might overlap with that of patients without HIV. Moreover, no significant AIDS-related infections or complications occurred (9).

In conclusion, we describe here the first case of a heart transplant in an HIV+ patient successfully carried out in Europe. We suggest that HIV infection, at least in CDC category A/WHO stage 1 patients, should no longer be viewed as an absolute contraindication to heart transplant.

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LETTER TO THE EDITOR

AZITHROMYCIN ANAPHYLAXIS IN CHILDREN

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Allergic reactions associated to the use of macrolides are uncommon; in particular only two cases of anaphylaxis with erythromycin and clarithromycin have been reported to date. The aim of this study was to investigate macrolide-induced anaphylaxis. Between December 2007 and December 2011, 136 consecutive children were referred to the Allergy Unit of A. Meyer Children's Hospital because of a past history of reactions to macrolides. Allergy work-ups were carried out according to the European Network for Drug Allergy protocol. Anaphylaxis was diagnosed according to the clinical criteria proposed by Sampson et al. and graded according to Brown SGA et al. Sixty-six out of 136 patients completed the allergologic work-up and among them we investigated three cases of anaphylaxis due to azithromycin which included one child with anaphylaxis to both clarithromycin and azithromycin. In two of the children with anaphylaxis, the diagnosis was only confirmed with the skin prick test, the third was positive to the Intradermal Test. The azithromycin allergy shows a surprisingly high sensitivity to the *in-vivo* tests. Moreover, this study shows that cross-reactivity may occur between different macrolidic molecules; it has even been suggested that macrolide allergies are unlikely to be class allergies.

Macrolides are characterized by their lactonic ring with two osidic chains (1), and are classified according to the number of carbon atoms in their cycle: 14-membered macrolides (erythromycin, troleandomycin, roxithromycin, dirithromycin, clarithromycin), azithromycin, which is the only 15-membered one, and 16-membered macrolides (spiramycin, josamycin, midecamycin). They act on bacterial growth by inhibiting ribonucleic acid (RNA)-dependent protein synthesis (2). Allergic reactions associated with the use of macrolides are uncommon and occur in 0.4-3% of treatments (1). Erythromycin has been most frequently described

among them as the cause of immediate IgE-dependent hypersensitivity reactions, followed by spiramycin and azithromycin (3). Immediate hypersensitivity reactions occur within the first hour after the last drug intake and are commonly manifested as urticaria (3). Delayed hypersensitivity reactions occur at any time between 1 h and 72 h after the last drug intake (4). According to literature, spiramycin may provoke urticaria, Schoenlein Henoch purpura, rhinoconjunctivitis and occupational asthma (5-7).

Clarithromycin is the most frequently prescribed oral macrolidic antibiotic among children (8) and it has been reported as the cause of IgE-

Key words: anaphylaxis, azithromycin, children, macrolides

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mediated bronchospasm, leucocytoklastic vasculitis, Schoenlein Henoch purpura and maculopapular exanthemas (9-11).

In studies only two cases of anaphylaxis to erythromycin (12) and clarithromycin (13) have ever been discussed. Azithromycin anaphylaxis has never been discussed before now. The aim of this study is to investigate macrolides-induced anaphylaxis.

MATERIALS AND METHODS

Between December 2007 and December 2011, 136 consecutive children (age range: 1-17 years; mean age: 8.5) were referred to the Allergy Unit of A. Meyer Children's Hospital because of a history of past reactions to macrolides. All anamnestic reactions were evaluated by the authors and had occurred during the previous year.

Anaphylaxis was diagnosed according to the clinical criteria proposed by Sampson et al. (14) and was graded according to the severity grading system by Brown SGA et al. (15).

Allergologic work-ups were performed according to the European Network for drug allergy protocol (Table I) (16). Skin testing with clarithromycin and azithromycin was performed on the first day. The concentrations used for the prick and ID tests respectively were 50 mg/ml and 10^{-2} - 10^{-3} in normal saline for clarithromycin and 100 mg/ml and 10^{-4} in normal saline for azithromycin (17).

We prepared clarithromycin and azithromycin no more than 2 hours before testing using the powder for i.v. administration. One drop of all of the above reagents were initially tested on volar forearm skin with the skin method at increasing concentrations and reactions were considered positive when a wheal larger than 3 mm in diameter with surrounding erythema appeared 10 min later.

After the prick tests, 0.03 ml of the reagent solution was injected intradermally on volar forearm skin. Readings

were made 20 min and 24, 48, 72 h after the injections. The results were considered positive when an increase larger than 3 mm in the initial wheal diameter appeared along with erythema. Positive checks for prick and ID tests were performed with histamine (at 10 and 1 mg/ml, respectively). Normal saline was used as a check for negative prick and ID tests. These tests were also administered with clarithromycin and azithromycin to 20 healthy children, who had previously tolerated clarithromycin, and ten who had also previously tolerated azithromycin.

Drug-specific IgE was determined by using a Radio Immuno Assay (RIA). According to Baldo and Harle's method (18), the solid phase was obtained by coupling the drug (clarithromycin and azithromycin) to sepharose epoxyactivated 6B through a spacer arm. Results are expressed as the percentage of radioactivity in the detecting antibody bound to the solid phase in relationship to the total amount of radioactivity introduced. Values of at least 3 times the level of normal serum (a pool of two sera of healthy, non atopic individuals) were considered positive.

Control subjects showed no positive reactions to either antibiotics skin tests. The oral provocation test (OPT) was performed as an open challenge with the culprit drug in all of those patients with negative *in vivo* tests.

Informed consent was obtained from all parents.

RESULTS

All 136 anamnestic reactions were collected by the authors: 93 (68.4%) reacted to clarithromycin [28 immediate and 65 delayed reactions (IR, DR respectively)]; 48 (35.3%) to azithromycin (10 IR and 38 DR); 6 (4.4%) to erythromycin (1 IR and 5 DR); 3 (2.2%) to roxithromycin (1 IR and 2 DR); 2 (1.5%) DR to rokitamycin. 16 (11.8%) out of 136 children reported reactions to more than

Table I. Clinical and immunological results in the three patients with anaphylaxis.

Patients	Age	Atopy	Latency	Time (m)	Route of Administration	Anamnestic reaction	SPT 100 mg/ml (w/e)	ID 10^{-4} mg/ml (w/e)	Sepharose 6B epoxyactivated	Total IgE (KUA/L)
Pt. 1	8	no	30'	4	i.v.	u/a/v	3/10	6/20	pos	276
Pt. 2	9	yes	<60'	4	o	a/w/d	3/10	4/6	pos	3304
Pt. 3	7	yes	3'	36	o	w/u/a	0/0	6/20	pos	338

Patient (Pt.)1,2,3: patient's number; m: months; w/e: wheal/erythema; i.v.: intravenous; o: oral; u: urticaria; a: angioedema; v: vomit; w: wheezing; d: dyspnoea; SPT: Skin Prick Test; ID: Intradermal Test; neg: negative; pos: positive

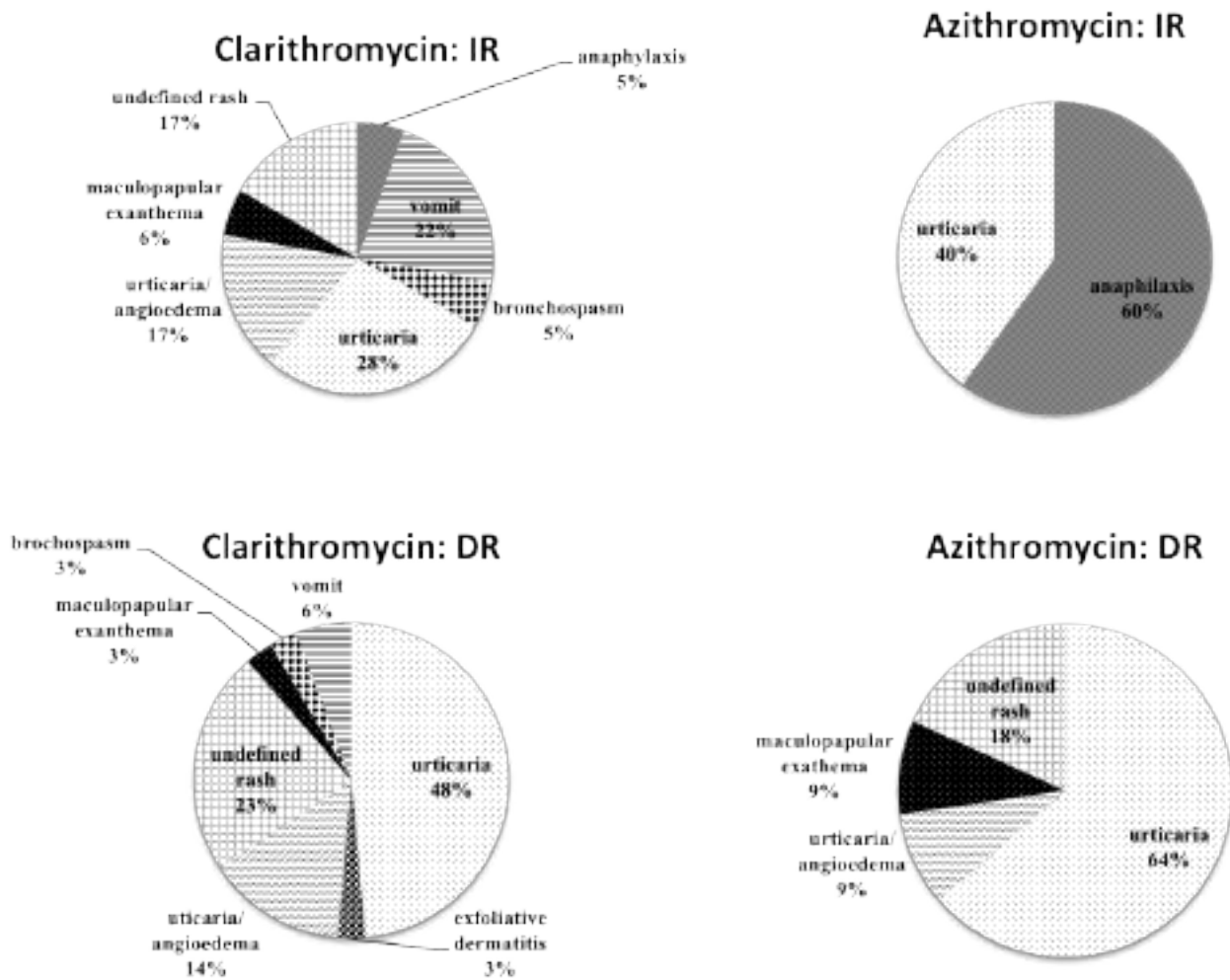


Fig.1. Symptoms for IR and DR of the studied population.

one type of macrolidic molecule. Most of them reported cutaneous symptoms (53.7% urticaria; 19% maculopapular exanthemas; 16% angioedema) and 3 children had anaphylaxis (2 to azithromycin and 1 to both azithromycin and clarithromycin) graded as moderate [Grade 2 according to Brown SGA (15)].

A total of 66 out of 136 patients completed the allergologic work-up, and among them only 22 patients had a history of IR (17 reacted to clarithromycin; 4 to azithromycin and 1 to both azithromycin and clarithromycin) and 44 patients had a history of DR (32 reacted to clarithromycin; 9 to azithromycin and 3 to both azithromycin and clarithromycin). In Fig. 1 we provide breakdown of symptoms for IR and DR of the 66 patients who underwent complete drug evaluation.

In the subgroup of 22 patients with IR the diagnosis was confirmed in 4 children (18.2%): three of whom with a history of anaphylaxis (2 to azithromycin and 1 to both azithromycin and clarithromycin) and one child with a history of clarithromycin urticaria. Specific IgE detection was performed in all 22 patients with 16 negative and 6 positive results. There were two false positive results among the positive results because of a negative OPT with the culprit drug (in particular a false positive result with clarithromycin and one with azithromycin antibiotic).

Among the IR obtained, there were 3 positive SPT results and 4 positive ID tests: 3 patients (2 with azithromycin anaphylaxis of which one is the subject with a history of reaction to both azithromycin and

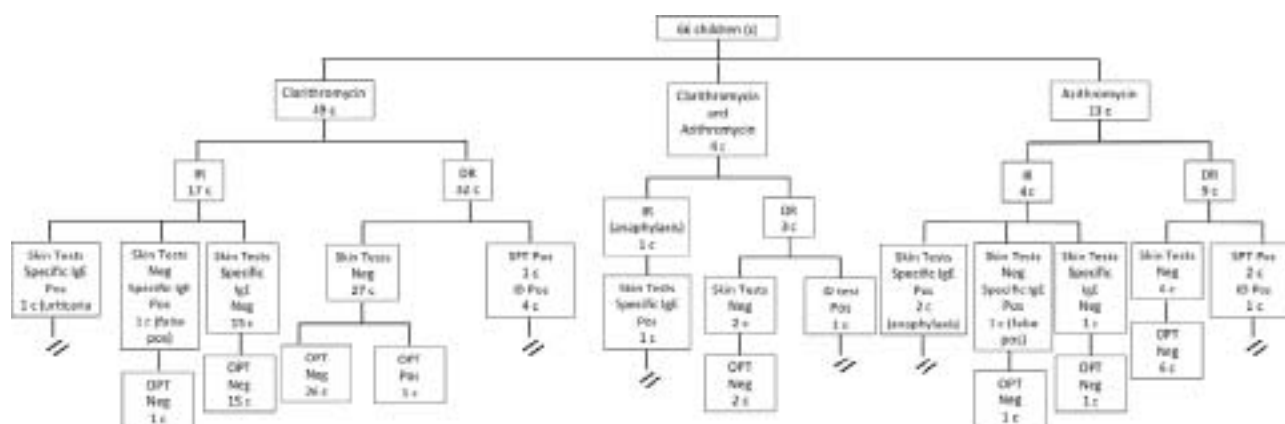


Fig. 2. Results of the allergy work-ups. (Skin tests: SPT and ID tests).

clarithromycin and 1 with clarithromycin urticaria) resulted positive to the SPT and ID tests and 1 with azithromycin anaphylaxis resulted with only 1 positive ID test.

Table I gives a brief description that includes the age of the patients treated and symptoms of azithromycin anaphylaxis. Patient 1 had anaphylaxis after the second dose of i.v. azithromycin antibiotic that was prescribed to treat a fracture of humerus. Before and after this episode, Patient 1 had tolerated roxithromycin antibiotic. Patient 2 had anaphylaxis after the second dose of azithromycin antibiotic that was prescribed to treat bronchitis. The same reaction occurred after the third dose of clarithromycin antibiotic about a year before the reaction to azithromycin.

Patient 3 had anaphylaxis to the first dose of azithromycin antibiotic that was prescribed to treat bronchitis. In this case the parents did not remember whether the antibiotic had already been used in the past.

The OPT (1/10-2/10-7/10 of the therapeutic dose, calculated according to each patient's weight, were administered every 20 min) was carried out on the 18 children (16 of whom with a history of a past reaction to clarithromycin and 2 to azithromycin) with negative cutaneous tests and resulted negative in all of them (Fig. 2). Thirty-five out of 44 children with a history of DR underwent the OPT with the culprit drug and a positive result was obtained in just one case (2.8%) (occurrence of late generalized urticaria after the last clarithromycin intake). Three patients resulted positive to the SPT, with azithromycin in

two cases and clarithromycin in one case, apart from their history of late reactions, and 6 patients resulted positive to the ID tests [in particular 2 were positive to azithromycin (of whom one child had a history of DR to both azithromycin and clarithromycin) and 4 were positive to clarithromycin]. These 9 patients refused to be provoked with the culprit drug because of the positivity of the skin tests (Fig. 2).

DISCUSSION

We investigated 66 patients with suspected allergy to macrolids (in particular, clarithromycin and azithromycin). The low incidence of patients evaluated compared to the total number 136 of those referred to our center may be due to the following factors: (a) some patients coming to our office from outside the region for asthma or eczema were found to have a history of drug allergy and hence decided to undergo drug allergy evaluation in a hospital closer to their home considering that the drug allergy work-up is time consuming; (b) in many cases the patients tolerated other antibiotics and refused to undergo drug allergy evaluation.

According to our results the two antibiotics (i.e. clarithromycin and azithromycin) cause almost the same spectrum of clinical reactions regarding both the IR and the DR, although azithromycin appears to be responsible for serious reactions more often than clarithromycin.

It is interesting to note that all three children with azithromycin-induced anaphylaxis in this study showed positive results in allergologic work-ups;

in particular, they were positive in two cases to the SPT resembling what we had previously described in cefaclor among beta-lactams (19). We performed ID tests in those patients where SPT was positive because of the weak positivity of azithromycin, in terms of wheal diameter, in comparison to the positive control and because of the lack of experience on skin tests with azithromycin in pediatric age. In patients with a history of anaphylaxis that could be dangerous, we decided to strictly observe the two children for the next two hours in a medical setting. In all children with IR we measured the specific IgE in the serum but this did not give us additional valuable information in respect to skin tests in the allergy work-ups.

One out of 3 patients (Patient 2) had severe reactions with both clarithromycin and azithromycin. Such a state of cross-reactivity was studied and confirmed by *in-vitro* and *in-vivo* tests including clarithromycin (both clarithromycin specific IgE in the serum and immediate reading of clarithromycin ID test at 0.05 mg/ml concentration resulted positive). Lastly, the third patient (Patient 3) had never used clarithromycin, yet resulted positive in the skin tests. An OPT is mandatory in order to exclude or confirm any cross-reactivity between clarithromycin and azithromycin. At the moment we are still waiting for parental consent.

This is the first study discussing anaphylaxis to azithromycin. This antibiotic represents 8.8% of all drugs prescribed in pediatric age (8). Moreover, even if clarithromycin is most frequently the cause of hypersensitivity reactions (probably because it is most frequently prescribed among macrolides), the frequency of anaphylaxis seems to be higher with azithromycin (30%). Finally, this study brings to the surface some new information regarding macrolide allergies because 2 out of 3 children studied tested positive to both clarithromycin and azithromycin. Thus, even if it has been suggested that macrolide allergies are unlikely to be class allergies (1), cross reactivity may occur between different macrolidic molecules at least regarding anaphylaxis.

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LETTER TO THE EDITOR

POMPHOLYX OF THE HANDS AFTER INTRAVENOUS IMMUNOGLOBULIN THERAPY FOR CLINICALLY ISOLATED SYNDROME: A PAEDIATRIC CASE

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Pompholyx is a common eruption of small vesicles on the palms, soles, and/or lateral aspects of the fingers. It has a multifactorial etiology, including genetic determinants, allergy to metals, and id reaction; rarely it is a drug-related side effect. We report a paediatric case of pompholyx of the hands related to the intravenous immunoglobulin (IVIG) therapy for Clinically Isolated Syndrome (CIS). A 10-year-old boy, received an IVIG therapy (Venital®, Kedrion Spa, Italy) at a dose of 400 mg/kg daily for five days. The fifth day of IVIG infusion, a symmetrical vesicular eruption appeared on the palms of the hands and on lateral aspects of the fingers. The lesions improved with application of topical steroids in few days. The mechanism of induction of pompholyx by IVIG therapy is unknown. A review of the Literature suggests the hypothesis that dyshidrotic eczematous reactions may be related not only to the type of IVIG, to the dose and the rates of infusion, but also to an allergic response to excipients and preservatives contained in the drug, probably elicited by an underlying neurological disease in some cases.

Pompholyx is a common, acute eruption of small (1-2 mm) vesicles on the palms, soles, and/or lateral aspects of the fingers, usually with a symmetrical distribution. It more often occurs in spring and fall, and generally has a self-limited course. Even if pathogenesis is not completely known, pompholyx has been regarded as a disease with a multifactorial etiology, including genetic determinants, allergy to metals, id reaction, and, rarely, it may be photo-induced or drug-induced (1, 2). Management of pompholyx needs to be adapted to etiology and includes local and systemic therapies, such as corticosteroids, calcineurin inhibitors, bexarotene gel, retinoids, and antihistamines (1).

We report a paediatric case of pompholyx related to the IVIG therapy for Clinically Isolated Syndrome

(CIS). CIS is the term to describe a neurological attack, lasting at least 24 h, characterized by optic neuritis, internuclear ophthalmoplegia, or partial myelitis, and it is associated with a major risk of developing multiple sclerosis (3).

Case history

A 10-year-old boy with acute onset of dizziness, diplopia, right eyelid ptosis, and divergent strabismus of the right eye was hospitalized in the Department of Paediatrics, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy. His past medical history was unremarkable. Viral serologic analyses for the detection of cytomegalovirus, Epstein virus and hepatitis virus were negative. Magnetic resonance imaging revealed findings suggestive

Key words: pompholyx, IVIG therapy, Clinically Isolated Syndrome, paediatric patient

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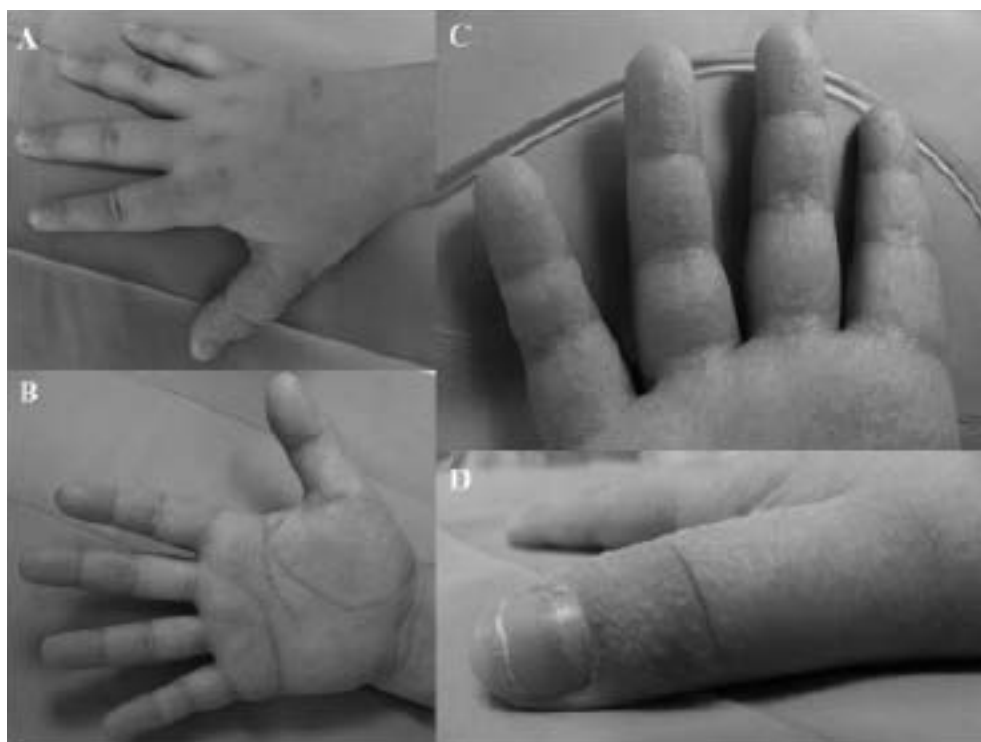


Fig. 1. Deep-seated vesicles on non-erythematous bases spread on the dorsa of the hands (A), on the palms (B, C) and on the lateral aspects of fingers (D).

of demyelination. A diagnosis of CIS was made. Intravenous immunoglobulin (IVIG) therapy (Venital®, Kedrion Spa, Italy) at a dose of 400 mg/kg daily for five days was started, with a subsequent improvement of neurological manifestations. The fifth day of infusion of IVIG therapy, a symmetrical, 1 to 2 mm, deep-seated, vesicular eruption on non-erythematous bases on the dorsa of the hands (Fig. 1A), on the palms (Fig. 1 B, C), and on lateral aspects of the fingers (Fig. 1D) appeared. The patient complained of intense itching. Considered the clinical features, a diagnosis of pompholyx was made. As personal and family history for dyshidrosis, atopy and allergy, fungal detection, and viral serologic analyses were all negative, IVIG-induced pompholyx was suspected. A steroid topic therapy with betamethasone valerate 0.1% cream was started leading to rapid improvement of the lesions, and complete recovery within a few days. At the follow-up after three months, complete clinical remission was reported. Furthermore, allergologic

investigations (skin prick test and patch-test) were performed and proved negative.

DISCUSSION

Over the past three decades, IVIG has become a valid treatment option for many pathological conditions ranging from primary and secondary immunodeficiencies, autoimmune diseases, neurological disorders, graft-versus-host disease to infectious and infection-related diseases (such as Kawasaki disease, sepsis, septic shock, and toxic shock syndromes) (4). Recently, there has been also evidence that IVIGs are a successful treatment for immune-mediated neurologic diseases, in particular demyelinating conditions, such as CIS (5). Cutaneous adverse effects of IVIG therapy are rare, especially in childhood, with an estimated general occurrence between 0.4 and 6% of treated patients. They include mainly urticaria, erythema multiforme, and eczematous reactions, in particular pompholyx

(6). The mechanism of induction of pompholyx by IVIG therapy is still poorly understood (7). Sorensen et al. (8) hypothesized that the occurrence of IVIG-induced eczematous reactions may be mainly related to the administration of high doses of IVIGs, the rapid infusion rates, and/or the underlying diseases (multiple sclerosis, in this case). The authors hypothesise that the administration of high doses of IVIG at a fast rate of infusion may induce the accumulation of substances toxic to the skin (8). Also the type of diseases for which IVIGs are used may have a role. In fact, Vecchietti et al. (2) assessed that neurologic disorders, frequently associated with immunological alterations or viral infections, may predispose the occurrence of pompholyx related to IVIG therapy. The authors noticed that most of the cases of IVIG-related pompholyx reported in the literature occurred among patients who had received IVIG for neurologic affections (31/37 cases described in this review), although considering the bias of more frequent use of IVIG therapy by neurologists (2). In addition, it should be pointed out that IVIGs for neurological diseases are generally administrated at higher doses than those used for immunodeficiency disorders (6).

Furthermore, Gerstenblith et al. (6) reported that several types of IVIGs correlate with a different risk of occurrence of pompholyx. In particular, Sandoglobulin® has been associated with a major risk of pompholyx compared to Gammagard® and Tegeline®. Since these IVIGs are different from each other for the content of IgA and for the amount and typology of excipients and preservatives (9), we could hypothesise that the occurrence of IVIG-induced pompholyx might be correlated not only to IVIG types, to the dose and the rates of infusion, but also to an allergic response to excipients and preservatives contained in the drug, probably elicited by an underlying neurological disease in some cases.

In conclusion, we report a case of IVIG-induced pompholyx of the hands in a pediatric patient because it is a rare finding in childhood, and to the best of our knowledge it is the first case of an IVIG Venital®-induced side effect. It is possible that CIS may have a role in predisposition of this reaction, as hypothesised by Gerstenblith et al. (6) even if further studies, including studies of pharmacogenomics, are necessary for an adequate comprehension of the pathogenic mechanisms that underlie this complex

adverse skin reaction.

Although pompholyx is generally successfully treated, dermatologists and paediatricians should be aware of this possible adverse effect of IVIG therapy and remember that it often recurs with a worsening trend in some cases (6).

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LETTER TO THE EDITOR

SYSTEMIC NICKEL ALLERGY SYNDROME: EPIDEMIOLOGICAL DATA FROM FOUR ITALIAN ALLERGY UNITS

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The prevalence of nickel hyper-sensitivity varies widely in different countries, nevertheless it is the leading cause of contact dermatitis. The presence of nickel in the diet (mainly plant foods) in some nickel-sensitive subjects can provoke/aggravate eczema and systemic contact dermatitis as well as cause extra-cutaneous symptoms (respiratory, gastrointestinal, neurological). These symptoms, correlated to the ingestion of nickel-containing foods and beverages, in nickel patch test positive individuals, defines the so called "systemic nickel allergy syndrome (SNAS)", a condition successfully treated by oral desensitization. Although numerous studies have investigated the prevalence of contact nickel allergy or addressed the relationship between nickel intake and onset of systemic symptoms, to our knowledge no epidemiological studies have attempted to estimate the prevalence of SNAS. Therefore, we decided to evaluate consecutive patients (1,696), afferent to four allergy units in Sicily, a region of southern Italy, from October 2010 to March 2011. SNAS was confirmed in 98 patients (5.78%) of the 1,696 studied, suggesting that this clinical entity may be an emergent allergological condition rather than an occasional finding. The most common symptoms complained of in our population were cutaneous (51 patients), gastrointestinal (87 patients) and other systemic clinical manifestations (37 patients). Furthermore, 16 out of the 98 SNAS patients (16.3%) presented IgE-mediated food allergy with a statistically significant association ($\chi^2=16.950$; $P<0.0001$), therefore suggesting underlying cross-facilitating pathways. These findings need confirmation on wider populations but may help allergists to suspect, during common clinical practice, that cutaneous and extra-cutaneous symptoms may be referred to nickel intake and deserve specific in-depth investigation.

Nickel is a chemical element largely diffused in the environment (1). The classical presentation of nickel allergy is allergic contact dermatitis (2). However, in some sensitized subjects, nickel is able to elicit cutaneous symptoms irrespective of the direct skin contact (3). The presence of nickel in the diet of

some nickel-sensitive subjects can provoke flare-ups of previous eczematous lesions (4). This condition is named systemic contact dermatitis (SCD) (5).

Sensitization to nickel can also cause respiratory symptoms (occupational rhinitis and asthma), urticaria, angioedema, gastrointestinal symptoms

Key words: epidemiology, Food Allergy (FA), nickel, Systemic Nickel Allergy Syndrome (SNAS)

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(recurrent aphthosis, abdominal bloating and pain, diarrhoea and/or constipation, nausea and or vomiting, with endoscopic findings of chronic gastroduodenitis) and other aspecific systemic clinical manifestations including headache, chronic fatigue, post-prandial dyspnea, cystitis and/or vulvovaginitis, acne and iron deficiency anaemia.

These cutaneous and extra-cutaneous symptoms, when correlated to the ingestion of foods and beverages containing nickel, define the so-called "systemic nickel allergy syndrome" (SNAS) (6). This clinical condition has been treated successfully with nickel oral hyposensitizing treatment (NiOH) (7) which has also proved to influence the nickel-induced immunologic response (8).

The correct approach to the diagnosis of SNAS should mimic the natural exposure, thus should be supported by a positive oral provocation test with nickel-containing foods (such as vegetables) or with nickel-containing capsules, after obtaining a significant improvement with a low nickel diet (9). Patients can be investigated for SNAS only if nickel sensitization is confirmed by a positive patch-test (10).

Although some data concerning the correlation between the ingestion of foods containing nickel and the onset of cutaneous and gastrointestinal symptoms have been previously reported (11-14), the available information is insufficient to draw definitive conclusions about the epidemiology of SNAS. For this reason we conducted a cross-sectional study aimed at determining the prevalence of SNAS among the population of patients afferent to four allergy units in Sicily, also reporting the most common symptoms of SNAS presentation and the most common triggering foods.

MATERIALS AND METHODS

Patients

Our study included 1,696 consecutive patients afferent to four allergy units in Sicily, a region of southern Italy, between October 2010 and March 2011. All of them had been referred by their General Practitioners (GPs) for allergological evaluation and allergy tests if needed.

Written informed consent was obtained from all subjects enrolled in the study.

Methods

Following the collection of clinical history and

physical examination of all patients, allergological evaluations were carried out, including skin prick tests (SPT) with commercial extracts (Stallergenes, Saronno, Varese, Italy) for common inhalants (house dust mites, moulds, *Parietaria Judaica*, grass and olive pollens, dog and cat epithelia) and foods (milk proteins, egg yolk, egg white, cod, shrimp, *Anisakis Simplex*, peanut, hazel, walnut, soybean, tomato, wheat, celery, carrot, potato, bean, apple, peach, orange, strawberry) and patch-tests with the Standard European series (Lofarma S.p.A. Milan, Italy), according to the International Contact Dermatitis Research Group (ICDRG) guidelines.

The clinical suspicion of SNAS was defined by a positive nickel patch test and the improvement, after one month on a low nickel diet (Table I), of the previously mentioned symptoms. To confirm the diagnosis of SNAS, the patients also underwent an oral provocation test (OPT) with nickel sulphate capsules (Lofarma S.p.A., Milano, Italy) in an open challenge fashion. The maximum cumulative dose of 3.75 mg of nickel sulphate was chosen on the basis of previous studies showing that this amount is approximately 10 times greater than the estimated normal daily dietary intake of nickel and therefore it is expected to cause flare-up reactions in most of nickel-sensitive individuals (13). Nickel sulphate was given at the dose of 1.25 mg the first day. In absence of symptoms two further single doses of 1.25 mg were given on the second day.

The skin status and the global clinical condition were evaluated and recorded 24 h after the challenge. A positive response was defined as the occurrence of cutaneous eruptions of previously unaffected skin, flare-up at previous sites of ACD (including the flare-up reactions of previously positive patch test sites), urticaria and/or angioedema and any other systemic manifestation, such as headache, marked fatigue and gastrointestinal symptoms (heartburn and abdominal distension, abdominal pain, diarrhoea and/or constipation, nausea and/or vomiting).

Statistics

Data are reported as mean $\pm$ SD. Differences between categorical groups were analyzed by the Pearson *Chi-square* (χ^2) test. In the statistical analyses the level of significance was set at $P=0.05$.

RESULTS

A total of 1,696 patients (mean age: 31.21 ± 12.45 ; 1,123 females and 573 males) afferent to four allergy units were included in the study; 697 patients (41.10% of total) were affected by rhinoconjunctivitis, 272 (16.04%) by asthma, 109

Table I. *Low nickel diet.*

FOOD ALLOWED	
SPICES AND DRESSINGS	Paprika, Pepper, Olive oil, Vinegar, Mayonnaise, Tomato sauce, Salt
DRINKS	Water, Fizzy drinks, Beer (not canned), Coffee, Wine
CEREALS	Plain flour, Bread, Pasta, Pizza, Rice
VEGETABLES AND FRUIT	Citrus fruits, Bananas, Apples, Melons, Peaches, Cucumbers, Aubergenes, Peppers, Potatoes, Garlic, Pumpkins, Courgettes
FISH	Swordfish, Tuna
SWEETS	Choux-pastry, Biscuits, Custard, Honey, Pastry, Sponge cakes, Sugar
OTHER	Meat, Eggs, Ham, Salami, Dairy products
FOOD NOT ALLOWED	
VEGETABLES	Asparagus, Broccoli, Artichokes, Carrots, Cabbage, Cauliflower, Onions, Fennel, Mushrooms, Lettuce, Tomatoes, Rocket salad, Celery, Spinach, Brussel sprouts
FRUIT	Apricots, Pineapples, Watermelon, Avocado, Cherries, Figs, Berries, Kiwi, Pears, Plums, Grapes
HERBS AND DRESSINGS	Bay leaves, Basil, Marjoram, Parsley, Ketchup, Seed oil, Mustard
CEREALS	Wholemeal products, Buckwheat Oat, Barley, Maize, Malt, Rye
LEGUMES	Lentils, Peas, Beans, Green beans
SEAFOOD	Cod, Shell-fish, Salmon, Octopus, Squid, Plaice
CHOCOLATE	All chocolate
NUTS	All
DRINKS	Tea

(6.43%) by food allergy (FA), 49 (2.89%) by ACD, 143 (8.43%) by adverse drug reaction (ADR), 30 (1.77%) by chronic idiopathic urticaria (CIU) and 298 (17.57%) by other non-allergic conditions. The presence of SNAS was clinically suspected in 108 patients (6.4%), all with a positive patch test to nickel and symptom improvement after one month on a

low nickel diet. The diagnosis was confirmed by a positive response to the OPT with nickel sulphate in 98 (5.78%) patients; the eliciting dose was 1.25 mg in 76 (77.5%) and 3.75 mg in 22 (22.5%) subjects, respectively. The 98 patients with SNAS were 88 females (89.8%) and 10 males (10.2%), mean age 36.8 ± 11.75 years.

Table II. Symptoms occurred in the 98 SNAS patients.

CLINICAL MANIFESTATIONS	N°	%
Meteorism	66	67.3
Bloating	56	57.1
Flare up of ACD	33	33.7
Headache	33	33.7
Heartburn	37	37.6
Recurrent abdominal pain	30	30.6
Urticaria and/or angioedema	23	23.5
Diarrhoea	16	16.3
Constipation	16	16.3
Systemic dermatitis	15	15.3
Migraine	7	7.1
Rosacea	6	6.1
Acne	5	5.1
Iron deficiency anaemia	1	1.0

Some allergic co-morbidities could be found in subjects with SNAS: 37 subjects (37.8%) also had allergic rhinoconjunctivitis, 10 (10.2%) had allergic asthma and 16 (16.3%) were diagnosed with an IgE-mediated food allergy (with positive history and skin tests) with a statistically significant association to SNAS ($\chi^2=16.950$; $P<0.0001$).

SNAS correlated symptoms are reported in Table II, triggered by a quite wide range of foods (Table III). Gastro-enteric symptoms like meteorism and bloating were very commonly correlated to SNAS, followed by flare-up of ACD, headache, heartburn, recurrent abdominal pain, urticaria and/or angioedema, while diarrhoea, constipation, and SCD occurred less frequently. Few patients complained of migraine, rosacea, acne, iron deficiency anaemia.

Legumes and tomatoes were the most common triggering foods, followed by cocoa and some vegetables like lettuce, broccoli, cauliflower and onions. The intensity of nickel patch test reactions in these patients was “+” in 4 subjects, “++” in 58 and “+++” in 36 but the grade of reactivity to nickel patch-test did not correlate to the intensity of SNAS symptoms. In some patients a concomitant positivity to other haptens, especially to cobalt chloride (16 patients), was found.

DISCUSSION

Although numerous studies document the prevalence of ACD to nickel, to our knowledge

Table III. Foods that triggered symptoms in the 98 SNAS patients.

FOOD	Patients
Lentils	51
Peas	42
Tomato	40
Beans	38
Green beans	37
Nuts	30
Cocoa	26
Lettuce	23
Broccoli	19
Cauliflower	15
Onions	15
Mushrooms	15
Tea	14
Whole wheat bread	13
Corn	13
Cabbage	13
Canned food	12
Mussels	12
Leavened food	11
Pears	11
Carrots	8
Figs	7
Asparagus	6
Shrimp	5
Liquorice	5
Prunes	5
Spinach	5
Cod	4
Apricots	3
Lobster	3
Buckwheat	2
Oysters	2
Flour	1
Oregano	1
Potatoes	1
Baked ham	1
Celery	1
Soy	1

no epidemiological study so far has addressed the issue of estimating SNAS incidence. In our study, SNAS was diagnosed in 98 patients out of 1,696; the

prevalence of SNAS in our sample was calculated as being around 5.78%, mostly women.

The main SNAS symptoms reported by our patients were gastrointestinal and cutaneous, apart from headache, and SCD which was present in 15.3% of patients; a similar incidence of nickel-induced SCD was reported to occur in 10% of patients consuming food with high nickel content (15).

The high prevalence of gastrointestinal symptoms in our SNAS population is supported by the findings of Di Gioacchino et al. (16) who observed a marked inflammatory infiltrate, mainly lymphoplasmacellular, associated to duodenum villus deformation and deepening of the crypta, in patients with nickel related ACD after an oral nickel challenge. Similarly, Cazzato et al. documented a nickel-related pro-inflammatory status at the intestinal mucosa in SNAS patients (17). The alteration of the intestinal barrier functions with an increased dietary antigen transportation, as well as exposure of dietary antigens to the mucosal immune system, may lead to the development of the dietary antigen-specific response (18) and could explain the high incidence of FA found in our SNAS patients.

Although the nickel content in individual foods can vary widely, a number of foods has been recognized as particularly rich in this metal, including peanuts, beans, lentils, peas, soybeans, oats, cocoa, chocolate, nuts, whole wheat, pears and mushrooms (19). Our study confirmed that fresh and dried legumes represent the most common foods triggering SNAS, followed by tomatoes, cocoa and nuts. Soy and oatmeal were rarely reported to cause symptoms, probably because of the low consumption of these foods in our region.

In conclusion, our data support the role of nickel-containing foods as potentially responsible for cutaneous and extra-cutaneous symptoms in nickel sensitized patients. These patients can be diagnosed with SNAS after a positive nickel patch-test result, symptom improvement following a low nickel diet and a positive result to a nickel OPT.

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LETTER TO THE EDITOR

THE GROWTH OF *STREPTOCOCCUS MUTANS* IN DIFFERENT MILKS FOR INFANT FEEDING

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After birth various bacterial species, mainly streptococci, colonize the oral cavity and are frequently isolated from carious lesions in children. Previous studies suggest that an earlier colonization of a child's mouth by cariogenic organisms might be related to a higher risk of caries. The aim of this study is to assess the influence of different milks for infant feeding on the growth of *Streptococcus mutans* (SM) colonies. Three human milk samples from three different mothers and five infant formulas were tested. To prepare the bacterial inoculum, SM was grown in Brain Heart Infusion broth for 18 hours at 37°C in microaerophilic atmosphere. The growth of SM was determined immediately after the inoculation of milks (T0) and after 24 hours (T24) of incubation. After 24 hours of incubation (ΔT = CFUs/ml at T24 – CFUs/ml at T0) the bacterial growth changes were different among milks. Among the complementary milks tested, ΔT of formulas supplemented with *Lactobacillus reuteri* and with *Bifidobacterium lactis* was lower than those of non-supplemented formula. In conclusion, on the basis of the reduced SM growth in milks supplemented with probiotics, we may speculate that these formulas have a preventive effect on the development of caries in children.

The mouth is an ideal ecosystem for the growth of microorganisms and the composition of its indigenous flora changes over lifetime.

During the prenatal period oral cavity is usually sterile and the bacterial acquisition is a complex process influenced by many perinatal factors such as pregnancy duration, type of delivery and birth weight (1). Soon after birth, the oral mucosa is colonized by different species of microflora, such as streptococci, staphylococci and Gram-negative aerobic bacilli (2). However, the balance of the oral ecosystem, similar to the gastrointestinal tract, relies on interaction between saprophytic and pathogenic bacteria (3). On

the other hand, any change of the oral microflora may allow pathogens to predominate and to cause diseases. This event increases the risk of developing one of the most common disease in children, such as the Early Childhood Caries (ECC) (4). This condition has an impact not only on oral health of young patients but also on global health (5). As a significant example, ECC can indirectly affect normal infant growth (6). Some bacteria are considered more caries-provoking than others, for example *Streptococcus mutans* (SM) is strongly associated with the pathogenic process of ECC (4). Previous studies suggested that an earlier acquisition of cariogenic organisms could

Key words: Milk formula, *Streptococcus mutans*, probiotics, caries, human milk

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be related with a higher risk of dental caries (7). In this perspective, the possibility of knowing the timing of *SM* colonization could play an important role in the ECC prevention. To date, few studies have investigated the oral colonization of *SM* in infants. Milgrom et al. and Wan et al. found that *SM* is present in an infant's mouth just before teeth eruption (8, 9). Although the *SM* colonization results from a complex interaction of bacterial factors with host factors, the infant feeding mode may be a proper intervention to promote the maintenance of oral health by contrasting early acquisition of *SM*. Over the past years, the clinical use of probiotic bacteria, also defined "good bacteria", has been extensively studied for their health-promoting effects (10), including beneficial impacts on oral health (11). The purpose of this study is to assess the influence of different milks for infant feeding on the growth of *SM* colonies and, hence, to evaluate the *in vitro* anti-cariogenic potential of different formulas.

MATERIALS AND METHODS

We performed a preliminary study, testing three human milk samples (HM1, HM2, HM3) from three different mothers, and five infant formulas (M1-5): an extensive hydrolyzed infant formula (M1; Althéra, Nestlé), a partially hydrolyzed infant formula (M2; Alfaré, Nestlé), a complementary milk from 6 months of age (M3; Nidina 2 liquid, Nestlé), a complementary milk from 6 months of age supplemented with *Bifidobacterium lactis* (M4; Nidina 2, Nestlé), a complementary milk from 6 months

of age milk supplemented with *Lactobacillus reuteri* (M5; Mio, Nestlé). Reconstituted milk formulas and human milks were analyzed in blind within 24 h.

Microbiological methods

SM ATCC25175^T (serotype c), was checked for purity on Columbia blood agar (Difco Laboratories, MD, USA) with 5% red sheep cells before the experiments. To prepare bacterial inoculum, *SM* was grown in Brain Heart Infusion (BHI) (OXOID Ltd., UK) broth for 18 h at 37°C in microaerophilic atmosphere (10% CO₂). After incubation, bacteria were harvested by centrifugation at 10,000 g for 10 min, washed twice and suspended in sterile saline solution (0.9% NaCl).

Before experimental contamination with *SM*, the milks for infant feeding were checked for pre-existent bacterial contamination. Lyophilized infant formulas were dissolved in sterile water following the manufacturer's instructions; the bacterial number present in the dissolved infant formulas and in human milk samples was evaluated using the colony forming units (CFUs) protocol. Briefly, serial dilutions of dissolved infant formula and human milk samples were plated into Columbia blood agar. The plates were incubated for 24 h at 37°C in microaerophilic conditions. After incubation, the number of the developed colonies was counted.

Infant formulas and human milks were inoculated with 10³ to 10⁶ CFUs/ml of the *SM* inoculum prepared as above described. The contaminated milks were incubated at 37°C for 24 h in microaerophilic conditions. The growth of *SM* was determined, as above mentioned, immediately after inoculation of milks (T0) and after 24 h (T24) of incubation. In control experiments, *SM* was cultured in BHI broth.

Table I. *P*-values of the comparisons among milks and between each milk and Brain Heart Infusion (Control) for ΔT .

	M1	M2	M3	M4	M5	HM1	HM2	HM3	Control
M1	-	0.0005	0.1611	<0.0001	<0.0001	0.0012	<0.0001	<0.0001	0.0013
M2	0.0005	-	0.0994	0.0066	0.0058	0.2446	0.0068	0.0058	0.5635
M3	0.1611	0.0994	-	0.0001	0.0001	0.0073	0.0001	0.0001	0.2186
M4	<0.0001	0.0066	0.0001	-	<0.0001	<0.0001	0.1801	0.0007	0.0032
M5	<0.0001	0.0058	0.0001	<0.0001	-	<0.0001	<0.0001	0.7743	0.0028
HM1	0.0012	0.2446	0.0073	<0.0001	<0.0001	-	<0.0001	<0.0001	0.1205
HM2	<0.0001	0.0068	0.0001	0.1801	<0.0001	<0.0001	-	0.0002	0.0032
HM3	<0.0001	0.0058	0.0001	0.0007	0.7743	<0.0001	0.0002	-	0.0028
Control	0.0013	0.5635	0.2186	0.0032	0.0028	0.1205	0.0032	0.0028	-

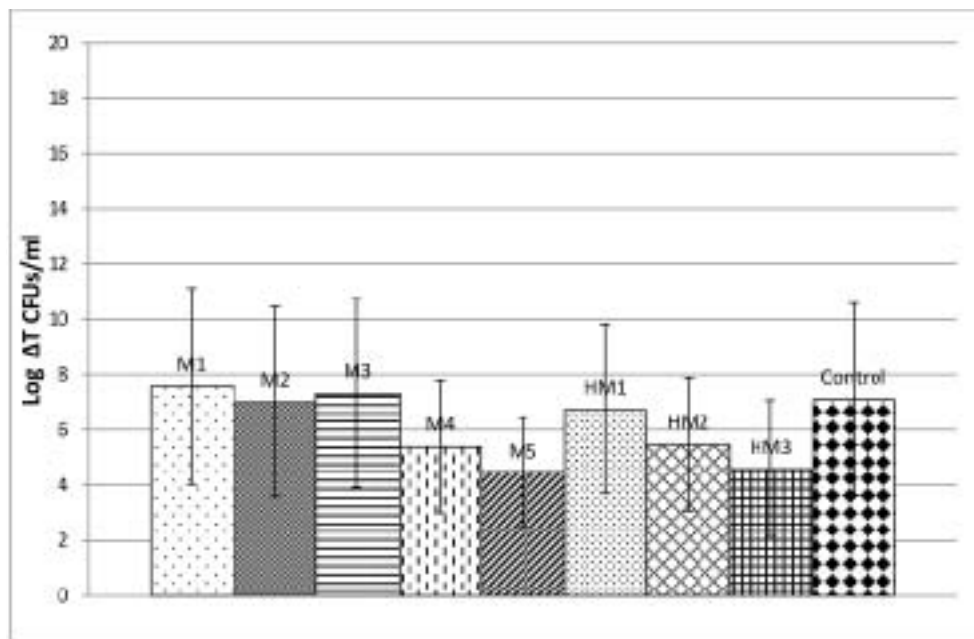


Fig. 1. ΔT of *Streptococcus mutans* in milk formulas. ΔT (CFUs/ml recovered at T24 – CFUs/ml inoculated at T0) of *Streptococcus mutans* cultured in BHI broth and in all the complementary milks included in the study. [Althéra, Nestlé (M1), an extensive hydrolyzed infant formula; Alfaré, Nestlé (M2), a partially hydrolyzed infant formula; Nidina 2 liquid, Nestlé (M3), a complementary milk from 6 months of age; Nidina 2, Nestlé (M4), a complementary milk from 6 months of age supplemented with *Bifidobacterium lactis*; Mio, Nestlé (M5), a complementary milk from 6 months of age milk supplemented with *Lactobacillus reuteri*; three human milk samples (HM1, HM2, HM3)].

CFUs were assessed at least three times in independent experiments in the inoculum and in milk samples after incubation.

Statistical analyses

Statistical analyses were performed with SPSS (Statistical Package of Social Sciences, Chicago, IL, USA) software version 19. Continuous data were expressed as means with SDs. Comparisons among CFUs inoculated and recovered and among the differences between CFUs recovered and inoculated (ΔT = CFUs/ml at T24 – CFUs/ml at T0) for each milk were evaluated using a *t*-test, after assessing for normality with the Shapiro-Wilk test. A General linear model for repeated measures was performed to investigate the change of CFUs/ml among milks over time. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Preliminary experiments were carried out to determine the number of bacterial cells in milks before the experimental contamination: any growth of microaerophilic bacteria was reported in infant

formulas while it was detected in the human milk samples (2.0×10^4 CFUs/ml for HM1, 15.1×10^3 CFUs/ml for HM2, and 1.3×10^3 CFUs/ml for HM3).

There was a statistically significant interaction between milk formulas and time on CFUs/ml ($F(9) = 7.624$; $p < 0.0005$). The bacterial growth changes after 24 h of incubation (ΔT) were different among milks (Fig. 1). *P*-values of the comparisons among milks and between each milk and Brain Heart Infusion (BHI) for ΔT are shown in Table I.

An increase in *SM* colonies compared to BHI were found out in M1, M3 and M2 while the lowest colonization was observed in HM3, M5, and M4. In particular, M4 and M5 respectively supplemented with *Bifidobacterium lactis* and *Lactobacillus reuteri* showed significantly lower colonization than the control sample.

DISCUSSION

The aim of this study was to compare the growth of *SM* colonies on different milks for infant feeding.

Results show that human milk samples have a lower amount of *SM* colonies if compared to control (BHI), except for HM1 sample.

It can be assumed that human milk could contrast the colonization of *SM* *in vitro*. These data are in line with recent findings reporting that human milk components, such as lactoferrin, IgA and IgG, might have an inhibitory effect on *SM* (12). Human milk also contains oligosaccharides (HMOs). It is generally accepted that HMOs have a prebiotic effect, mimicking the attachment sites for certain pathogens and blocking their adhesion, colonization and invasion (13). On the other hand, beneficial effects of human milk in contrasting ECC is controversy. Several studies have reported an inverse association between breastfeeding and ECC, while other studies have failed to show any highly beneficial association. A systematic review identifies possible risk factors for ECC in breastfeeding for longer than 1 year, as well as nighttime breastfeeding after teeth eruption, although methodological concerns and inconsistent definition of breastfeeding used in the studies examined make it difficult to draw definitive conclusion (14). Anticariogenic effect of human milk due to its concentration of calcium and phosphate has also been reported (15). Although our findings show a lower bacterial growth in human milks compared to control sample, these conclusion are limited due to the low number of samples analyzed.

By analyzing the infant formulas, we realized that supplementation with *Lactobacillus reuteri* and *Bifidobacterium lactis* is associated with a significant reduction of *SM* colonies if compared to the non-supplemented formula. From these results it is reasonable to affirm that probiotics may play a potential role in contrasting *SM* colonization. Little information is available about the relationship between probiotics and counts of *SM*. *In vitro* studies showed that probiotic strains may affect the oral ecology by preventing the adherence of other bacteria to saliva-coated hydroxyapatite and by modifying the composition of the salivary pellicle (16). Other mechanisms of action for probiotics on oral health are described in literature, including adhesion, co-aggregation, competitive inhibition, production of organic acids and bacteriocin-like compounds and immune-modulation (17). In particular, the enhanced resistance to oral infections

could be obtained through the interaction among probiotics, oral epithelium and saliva. Salivary proteins can modulate microbial colonization by stimulating their adhesion to epithelium surface and their metabolic activity. This cooperation may also promote the modulation of innate and adaptive host immune response (18). The beneficial probiotic effects also include an alteration of the composition and metabolic activity of host micro-flora at the specific location. Several studies have shown that the use of probiotics allows the replacement of cariogenic bacteria with those non-cariogenic (19). We can speculate that beneficial effect of probiotics is limited by the fact that probiotic bacteria are not able to permanently colonize oral cavity (20). To ensure a longer duration of probiotics in oral environment, it seems necessary to determine which is the most suitable vehicle. Short effect of probiotics in oral cavity may be contrasted through their continuous administration in infant formula, as a natural and long-term approach.

In conclusion, despite our results are limited by the low number of samples analyzed, they suggest that infant formula supplemented with probiotics may have an antimicrobial activity against *SM* and, consequently, a preventive effect on maintaining and promoting oral health by delaying *SM* colonization. Although human milk is the benchmark regarding each health outcome in the first months of life, these preliminary results identify an increased *in-vitro* capacity to reduce the growth of *SM* in milks supplemented with probiotics and encourage further large-scale studies on usefulness of probiotics to promote oral health.

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LETTER TO THE EDITOR

SACCHAROMYCES CEREVISIAE FUNGEMIA, A POSSIBLE CONSEQUENCE OF THE TREATMENT OF *CLOSTRIDIUM DIFFICILE* COLITIS WITH A PROBIOTICUM

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The yeast *Saccharomyces boulardii* is a biotherapeutic agent used for the prevention and treatment of several gastrointestinal diseases, such as diarrhoea caused by *Clostridium difficile*, in addition to the antibiotic therapy. In this study we report a case of *Saccharomyces cerevisiae* fungemia in a patient with *Clostridium difficile*-associated diarrhoea (CDAD) treated orally with *S. boulardii* in association with vancomycin. The identification of the *S. cerevisiae* was confirmed by molecular technique. Fungemia is a rare, but a serious complication to treatment with probiotics. We believe it is important to remind the clinicians of this risk when prescribing probiotics, especially to immunocompromised patients.

Fungal infections are an increasing cause of morbidity and mortality particularly in immunocompromised patients. Although most fungal infections are caused by *Candida* and *Aspergillus* species, over the past decade there has been increasing recognition of other opportunistic fungi (1, 2).

Clostridium difficile is a major cause of antibiotic associated diarrhoea within the hospital setting (3). The antibiotics most commonly used to treat *C. difficile*-associated diarrhoea (CDAD) include metronidazole or vancomycin, but vancomycin is superior for treating patients with severe CDAD (4). Probiotics represent a promising approach as an adjunctive therapy for CDAD. *Saccharomyces cerevisiae* is a well-known yeast used in the food industry and it has now been established that this yeast can cause different forms of invasive

infection. The use of the probiotics for prevention and treatment of acute or chronic diarrhoea has been controversial because there have been case reports of fungemia in both immunocompromised and immunocompetent patients (5). The mechanism of action of this probiotic includes the regulation of intestinal microbial homeostasis, the interference with the ability of pathogens to colonize and infect the mucosa, the modulation of local and systemic immune responses, the stabilization of the gastrointestinal barrier function and induction of enzymatic activity favouring absorption and nutrition (6). *Saccharomyces* fungemia is the most severe complication secondary to administration of the probiotics, especially in patients with an indwelling catheter (7). In this article, we described a case of *S. cerevisiae* fungemia in a patient treated orally with *Saccharomyces boulardii* in association

Key words: *Saccharomyces cerevisiae*, fungemia, *Clostridium difficile*, probiotics

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with vancomycin for *C. difficile* colitis.

MATERIALS AND METHODS

Patient

An 86 year-old man was admitted to the emergency department of Sant'Andrea Hospital in Rome for a possible *C. difficile*-associated diarrhoea due to an antibiotic treatment with ceftriaxone for an acute exacerbation of chronic obstructive bronchitis. A stool sample was positive for *C. difficile* toxin by enzyme-linked fluorescent immunoassay (ELFA) performed with the automated VIDAS instrument (bioMérieux, Marcy l'Etoile, France). Moreover, bacterial stool culture was positive on commercially available CLO agar plates (bioMérieux). Stool samples analyzed for enteric pathogenic bacteria and parasite were negative. Abdominal computed tomography showed pancolitis.

The patient was treated with oral vancomycin 500 mg four times daily, and also with a probiotic preparation, labeled Codex (Zambon Farmaceutici, Vicenza, Italy) and declared to contain 250 mg of lyophilized *S. boulardii*, three times daily for ten days, until clinical improvement. The subsequent microbiological control showed the negativity of the stool sample for *C. difficile* toxin. However, after 10 days of treatment, the patient presented fever and infectious parameters were elevated. Laboratory data showed a normal white blood cell count of 4.5 thousand/L (normal range, 4.30–10.8 thousand/L), a hemoglobin level of 9.7 g/dL (normal range, 13.0–18.0), a C-reactive protein greater than 3 mg/dL (normal range, 0.00–0.50) and procalcitonin level of 5.88 ng/ml (normal range <0.05).

Two sets (aerobic and anaerobic) of blood cultures with BacT/Alert System (BD Diagnostic Systems, Sparks, Md.) were performed from peripheral veins and from the indwelling catheter, previously positioned to perform fluid therapy. The catheter was removed and put in culture.

Isolation and antimicrobial susceptibility

The positive blood cultures were subcultured onto plate media selected in accordance with the Gram stain result, and identification of the *S. cerevisiae* was carried out using a combination of conventional and commercial methods API ID32C and VitekII system (bioMérieux). Antifungal susceptibility testing for amphotericin B, fluconazole itraconazole, voriconazole, and caspofungin was performed by E-test (Liofilchem®, Roseto degli Abruzzi, Teramo, Italy) against *S. cerevisiae*.

Amplification and sequencing

The identification of the *S. cerevisiae* was confirmed by molecular technique. A couple of specific primers

for 18S ribosomal RNA gene of *S. cerevisiae* were designed, 18S Forward TCCTGGTTCGCCAAGAGCCA and 18S Reverse GGCAGCAGGCGCGCAAATTA. The molecular analysis was carried out by PCR in a GeneAmp® PCR System 9700 (Applied Biosystems). The amplification was purified and then sequenced using BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems) as recommended by the manufacturer. The sequences were determined with an ABI Prism 3130XL Genetic Analyser (Applied Biosystems). The sequences were compared to reference data available in the GenBank database by using BLAST (basic local alignment search tool) that determined for the strain tested a correct identification for *S. cerevisiae* with a rate of 100% (278/278). Results were further aligned with a reference sequences (JX021607.1) using Navigator software (Applied Biosystems).

RESULTS

The result of microbiological analysis and the additional nucleotide sequencing reactions showed that the organism was *S. cerevisiae*. *S. cerevisiae* was isolated from both (peripheral veins and indwelling catheter) aerobic blood cultures, while the culture of the removed catheter was negative. No other pathogens were detected.

Susceptibility testing indicated that *S. cerevisiae* was susceptible to caspofungin and voriconazole but resistant to fluconazole, itraconazole and Amphotericin B. Therapy with caspofungin was initiated. Intravenous caspofungin (initial dose of 70 mg followed by 50 mg daily) was administered to our patient with a fast improvement within 24 hours (diminished fever and improvement of the infectious biochemical parameters).

DISCUSSION

Probiotics represent a promising approach as an adjunctive therapy for CDAD however, *Saccharomyces* fungemia is the most frequent complication secondary to administration of the probiotics especially in patients with an indwelling catheter (7).

Although *S. cerevisiae* is considered a safe and non-pathogenic agent, several reports show that this fungus may indeed cause severe infections including fungemia, endocarditis, pneumonia, liver abscess, peritonitis, and septic shock. Review of published

cases indicates that invasive infections with *S. cerevisiae* occur in immunocompromised patients, but also in relatively healthy individuals. The incidence of *S. cerevisiae* fungemia is unknown, although population-based studies suggest that it is responsible for 0.1%–3.6% of all episodes of fungemia (8-10), and several recent studies reported that *S. cerevisiae* fungemia had occurred in patients with a history of use of probiotics or evidence of nosocomial acquisition in an environment where probiotics were used (11-17). *S. cerevisiae* fungemia was first reported in 1970 in a patient with a prosthetic mitral valve (18), fifteen cases (16.3%) were diagnosed before 1990, although the incidence has significantly increased since the 1990s. In a review by Munoz et al. (19), 60 cases of *Saccharomyces* fungemia were reported from 1996 to 2004, and 54.6% of cases followed the use of probiotics by a median time of 10 days. Since 2005, other cases (20-25) of invasive *Saccharomyces* infections have been diagnosed and all patients had at least one condition facilitating the development of invasive fungal infection.

In this study we report a case of fungemia due to *S. cerevisiae* in a patient with CDAD and with insertion of a venous catheter admitted to the emergency department of Sant'Andrea Hospital in Rome. The origin of the fungemia is thought to be either a digestive tract translocation or a contamination of the central venous line by the infected hands of health workers (17). Contamination of the air, environmental surfaces, and hands following the opening of a packet suggests that catheter contamination may have been a source of infection. To prevent catheter contamination it is recommended that packets or capsules of *S. boulardii* be opened with gloves, outside the patient's room.

A ready and close cooperation between the clinician and the microbiologist allowed to identify the microbiological agent and then the target the therapy for the treatment of the sepsis yeast infection.

Most cases of *S. cerevisiae* fungemia related to oral administration of *S. boulardii* have been treated with amphotericin B or fluconazole. We treated the patient with caspofungin with excellent results, in agreement with those reported by Lolis et al. (26) in a previous paper.

In conclusion, this report confirms the risk of infection of *S. cerevisiae* fungemia, identification

confirmed by sequencing of the gene for rRNA, in a patient treated orally with probiotics. The need for stringent application of proper hygiene when using a probiotic preparation of this organism is required particularly for patients with a central venous catheter or in immunocompromised or critically ill patients.

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ERRATUM CORRIGE

Volume 26 No. 3, page 704, Acknowledgements section: grant no. PR01/09 should read RP01/09.

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