



Autonomic nervous system dysfunction and their relationship with disease severity in children with atopic asthma

Ozkaya Emin^{a,*}, Gursoy Esra^b, Demir Aysegül^c, Erenberk Ufuk^c, Sogut Ayhan^d, Dundaroz M. Rusen^e

^a Department of Pediatrics, Division of Pediatric Allergy, Bezmialem Vakif University, Istanbul, Turkey

^b Department of Neurology, Bezmialem Vakif University, Istanbul, Turkey

^c Department of Pediatrics, Bezmialem Vakif University, Istanbul, Turkey

^d Department of Pediatrics, Division of Pediatric Allergy, Regional Education and Research Hospital, Erzurum, Turkey

^e Department of Pediatrics, Division of Pediatric Emergency, Bezmialem Vakif University, Istanbul, Turkey

ARTICLE INFO

Article history:

Accepted 3 July 2012

Keywords:

Asthma
Autonomic nervous function
Children
Sympathetic skin response

ABSTRACT

The involvement of autonomic imbalance has been reported in the pathogenesis of allergic diseases. The aim of this study was to investigate the association between the clinical severity of childhood asthma with autonomic nervous system (ANS) dysfunction and to define whether the severity of asthma correlates with ANS activity. In this case–control study, we evaluated the ANS activity by testing heart rate variability (HRV) and sympathetic skin response (SSR) in 77 asthmatic children, age 7–12 yrs, who had no co-morbidity and compared them with 40 gender- and age-matched control subjects. According to the severity of their asthma, study subjects were further divided into three groups: I (mild asthmatics), II (moderate asthmatics), and III (severe asthmatics). Inter-group ANS scale scores differed significantly ($p < 0.01$) between Groups I and III and between Groups II and III. Combined use of HRV and SSR provides a higher degree of sensitivity for assessing disease severity in cases of pediatric asthma.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

In recent years, asthma has become the most common chronic childhood disease (Asher, 2008). An important feature of asthma is an exaggerated broncho-constrictor response to a wide variety of stimuli. This airway hyper-responsiveness can be attributed in part to an airway inflammatory response as markers for airway inflammation correlate with bronchial hyper-responsiveness. However, anti-inflammatory therapy merely reduces airway hyper-responsiveness, but does not abolish it; therefore, factors other than inflammation may contribute to airway hyper-responsiveness (Lundgren et al., 1988). Asthma is also associated with autonomic nervous system (ANS) imbalance, increased bronchial sensitivity to cholinergic constrictors, and possibly decreased sensitivity to β_2 -adrenergic dilators (Jartti, 2001). According to Meessen et al. (1996), several mechanisms may contribute to exaggerated cholinergic bronchospasm in asthmatics and the release of inflammatory mediators, such as airway epithelium damage due to viral infections, allergen stimuli, or various chemical and mechanical irritants (cold air and tobacco

smoke) when in close contact with sensory vagal nerve endings (Meessen et al., 1996). From a physiological perspective, parasympathetic innervation mediates both cholinergic contractions and non-cholinergic relaxation of the airway (Chesrown et al., 1980). Asthma presents either as a hyperfunction of the parasympathetic nervous system (PNS) in the airways or as an imbalance between the PNS and the sympathetic nervous system (SNS) (van der Velden and Hulsman, 1999). The hypothesis that PNS dysfunction is the cause of asthma is based on both clinical observation (Morrison and Pearson, 1991; Lin et al., 1998; Ducharme and Davis, 1998) and experimental research (Ollerenshaw et al., 1991). No studies have objectively investigated the relationship between the severity of asthma as an autonomic nervous system (ANS) dysfunction in childhood. With the introduction and use of modern-day laboratory testing for dysautonomia, we examined a series of children with asthma in an attempt to further elucidate the relationship between disease severity and ANS dysfunction.

2. Materials and methods

2.1. Ethical approval

The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice and was approved by the local Ethics Committee. Informed consent was obtained from the parents of all study participants.

* Corresponding author at: Bezmialem Vakif University, Department of Pediatrics, Division of Pediatric Allergy, Fatih, Istanbul, Turkey. Tel.: +90 532 442 42 22; fax: +90 212 621 75 80.

E-mail address: minozkaya@yahoo.com (O. Emin).

URL: <http://www.bezmialem.edu.tr/> (O. Emin).

2.2. Subjects

This study was conducted from December 2010 through June 2011 in the Pediatric Allergy-Pulmonology outpatient clinic at Bezmialem Vakif University Hospital. The sample population was comprised of 77 children (46 boys, 31 girls; aged 7–12 years) who had been recently diagnosed and followed up for asthma, but who had no other co-morbidities. The diagnosis and severity of asthma were defined according to guidelines of the American Thoracic Society ([American Thoracic Society, 1995](#)). Children were defined as asthmatics according to the following criteria: (a) recurrent episodes of at least 1 symptom of asthma: wheezing, breathlessness, and chest tightness, (b) improvement of at least 12% in baseline with forced expiratory volume in 1 second (FEV₁) after bronchodilator use, (c) a total serum Ig E level > 52 IU/mL on direct chemiluminescence, and (d) a positive skin test for at least 1 allergen. Informed consent was given by the parent of the patients and the children were provided education about the parameters of the study in accordance with the Declaration of Helsinki and Good Clinical Practice.

Subjects were divided into three groups according to their asthma severity scores (ASS) and forced expiratory volume in 1 s (FEV₁): Group I (mild intermittent) used neither inhaled nor oral steroids or leukotriene receptor antagonists, Group II (moderate) used inhaled steroids and salbutamol during attacks as well as a leukotriene receptor antagonists when needed, and Group III (severe asthma) used inhaled steroids, formoterol, montelukast, and salbutamol during attacks. An ASS scoring system was also implemented to better define participant responses: mild intermittent asthma = score I, moderate asthma = score II, and severe asthma = score III ([Santanello et al., 1997](#)). Children with asthma were evaluated according to their daytime symptoms of coughing, chest tightness, wheezing, sputum production, and breathlessness.

Control group subjects were selected from children admitted to the Bezmialem pediatric outpatient clinic for routine check-up who were free of physical signs and symptoms indicative of atopic diseases including allergic rhinitis, asthma, and atopic dermatitis.

2.3. Data collection

2.3.1. Lung functions studies

All children accepted in the study were able to perform forced expiratory maneuvers. Forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), forced expiratory flows at 25–75% of the vital capacity (FEF 25–75%), and peak expiratory flow (PEF) rates were measured with a dynamic spirometry [Vitalograph®; G.W. Berg-Co., UK]. The best of three successful maneuvers was recorded. After baseline measurement was established, a bronchodilation test with inhaled terbutaline was performed on all children. A repeat post-bronchodilator spirometry measurement was performed after 15 min. In the bronchodilation test, the limit of positivity was set at a $\geq 12\%$ increase in FEV₁ values and a 25% or greater increase in FEF 25–75% values ([National Heart, Lung, and Blood Institute, 2007](#)). The results were presented as percent of predicted value for gender, age, and height. The Polgar normal lung function test reference values were used to generate the predicted values ([Polgar and Promadhat, 1971](#)).

2.3.2. Skin prick tests

Skin prick tests (SPT) were performed according to a routine diagnostic procedure. Ten common aeroallergens with pollens, mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), molds, and animal dander were used. Negative (50% glycerin saline) and positive (0.1% histamine phosphate) controls were also tested. The skin tests were examined 15 min post exposure to the allergen and the resulting wheal and flare were

measured. A test was considered positive if the wheal was 3 mm greater than the negative control wheal.

2.3.3. Autonomic nervous system (ANS) tests

The ANS was tested in three ways.

1. Heart rate response to deep breathing was quantified. The patient was instructed in and allowed to practice breathing deeply and smoothly using inspiratory and expiratory cycles of 5 s each. The heart rate was recorded using an electrocardiogram monitor with electrodes placed at standard sites. Eight cycles of deep breathing (10 s each) were accomplished in the manner practiced. The computer measured the heart rate and the mean of the five largest consecutive responses to calculate the heart rate range to deep breaths (HRDB).
2. Heart rate responses to the *Valsalva maneuver* were recorded using an electrocardiogram monitor [Finapres, Englewood, CO, U.S.A.]. The patient blew into a tube connected to a manometer and maintained a pressure of 30 mm Hg for 10 s. Time-domain and frequency domain parameters of heart rate variability (HRV) were recorded and analyzed using the same device. The time-domain HRV parameters used to measured data in the study were (a) the standard deviation of all normal sinus R–R intervals (SDNN), (b) root mean square of successive differences between normal sinus R–R intervals (RMSSD), (c) the ratio of all R–R intervals to the height of the histogram created by charting all the RR intervals (HRV triangular index), and (d) number of R–R intervals exceeding 50 ms (SNN50 count). We also used power spectral analyses of heart rates with the parameters of low frequency (LF: 0.04–0.15); this measure is related to baro-receptor control and is dually mediated by both the parasympathetic and sympathetic systems. High frequency (HF: 0.15–0.5) bands were used to reflect respiratory sinus arrhythmia and thus cardiac vagal activity. The LF/HF ratio is the most important parameter for assessing the autonomic imbalance and this ratio was used to define imbalance in ANS for our study.
3. *Sympathetic skin response (SSR)*: We also measured SSR because of its reliability as an indicator of sudomotor function mediated by amylinic efferent C-fibers of the sympathetic nervous system ([Elie and Guiheneuc, 1990](#)). Sympathetic skin response (SSR) is a polysynaptic reflex and its final efferent pathway involves pre- and post-ganglionic sympathetic sudomotor fibers that ultimately activate the sweat glands through sympathetic outflow ([Shahani et al., 1984](#)). SSR testing was carried out according to the Technical Standards of the International Federation of Clinical Neurophysiology ([Claus and Schondorf, 1999](#)). The EMG was performed using a filter setting of 0.1–32 Hz with a sensitivity setting of 1 mV div⁻¹ and sweep rate of 5 s. Responses were recorded with superficial electrodes on the skin at an impedance of <5 k Ω . In each location, the mean latency and percentage of SSR (SSR%) were determined. The results of latency were classified into two groups as normal or abnormal (increased) latency with the latter being considered pathological. The amplitude was measured from the baseline to the negative peak and expressed in mV's. The response was considered absent if no consistent voltage change occurred using a sensitivity of 50 μ V per division after three trials at maximum intensity for the stimulus.

2.3.4. Statistical analysis

The study data were analyzed using SPSS (Statistical Package for Social Sciences) for Windows version 16.0. A *t*-test was used to compare continuous variables including age, weight, height, and body mass index (BMI) when the data were normally distributed and the ANOVA was used to compare means of three or more independent groups. Normality tests of variables were assessed by the one sample Kolmogorov–Smirnov test. Differences between groups were

Table 1
Demographics and clinical characteristics of patients and controls.

	Group I N = 23	Group II N = 33	Group III N = 21	Controls N = 40	p
Age, mean (yrs)	8.73 ± 3.7	8.2 ± 4.2	8.9 ± 3.9	8.5 ± 2.8	>0.05
Male (%)	14(60)	19(58)	13(62)	25(62)	>0.05
Height, mean (cm)	133.0 ± 3.2	134.4 ± 2.6	131.4 ± 2.4	134.1 ± 3.8	>0.05
Weight, mean (kg)	31.4 ± 1.2	32.8 ± 0.9	32.3 ± 1.1	33.8 ± 1.4	>0.05
Skin prick test positivity to mite, no. patients (%)	23(100)	33(100)	21(100)	–	>0.05
IgE (kU/L) (SD)	456.7 ± 45	468.7 ± 45	440.6 ± 31.5	120.7 ± 30.7	>0.05
Symptoms duration, mean (yrs)	5.3 ± 0.8	4.9 ± 0.8	5.7 ± 0.4	–	>0.05
BMI (kg/m ²) (SD)	14.52 ± 3.0	15.25 ± 2.1	15.13 ± 2.2	14.83 ± 1.9	>0.05
FEV1 (% predicted) (SD)	83.70 ± 15.2	76.20 ± 11.9	54.55 ± 12.3	89.80 ± 17.2	<0.05

BMI, body mass index.

Values are given as mean ± standard error of the mean.

evaluated with either the Kruskal–Wallis test or Mann–Whitney *U*-test. Chi-square analysis was used to compare categorical data. Inter-group logistic regression analysis was performed for the evaluation of sensitivity of the composite autonomic nervous scoring scale (CANSS) in predicting asthma severity. In all tests, $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Subjects

The sample for this study consisted of a total of 36 (56%) boys and 28 (44%) girls. According to the severity of their asthma, each subject was assigned to one of three groups: Group I (Mild Intermittent Asthma), Group II (Moderate Asthma), or Group III (Severe Asthma). The mean ages of Groups I, II, and III were 8.73 ± 3.7 , 8.2 ± 4.2 , and 8.92 ± 3.9 , respectively. Gender distribution in the study groups were as follows: Group I: 14 (61%) boys and 9 (39%) girls; Group II: 19 (58%) boys and 14 (42%) girls; and Group III: 13 (67%) boys and 8 (43%) girls. There were no significant differences between the groups for age, gender, or BMI ($p > 0.05$) (Table 1). When the asthmatic children were classified according to the severity of their asthma, age; gender; duration of the disease; and serum IgE levels were also found to be similar.

3.2. Measures of ANS tests

A comparison of the autonomic nervous system testing scores of the control subjects with those of patients with asthma revealed significant differences for HRDB and HRV ($p < 0.05$), but not for SSR ($p > 0.05$) (Tables 2 and 3, respectively). HRDB and HRV subscores represent primary dysfunction of the PNS. On the autonomic

scoring scale all scores in patient groups were statistically significant ($p < 0.05$) when compared with the control group (Table 2).

3.3. Factors associated with ANS test parameters

Autonomic nervous system testing scores between Groups I and III, and between Groups II and III for both the Kruskal–Wallis and Mann–Whitney *U*-tests test were $p < 0.01$. The sensitivity of ANS testing scores in predicting asthma severity was evaluated using logistic regression analysis (Table 4). The sudomotor subscores indicated primarily an abnormality of the SNS. The cardiovagal subscore indicates dysfunction of the PNS; while, the adrenergic subscore indicates dysfunction of the SNS. The composite autonomic nervous scoring scale (CANSS) is a composite of the above two subscores. No significant difference was found for CANSS when compared to the controls and Group I ($p > 0.05$). On the other hand, there was significant relationship between increases in CANSS and asthma severity, between Groups I and III, and between Groups II and III. The sensitivity of CANSS in predicting asthma severity was 78% between Groups I and II, 72.8% between Groups II and III ($p < 0.01$).

4. Discussion

To our knowledge this is the first study to assess HRV and SSR, parameters of ANS functions, for evaluation of disease severity in selected outpatient children with asthma. This study showed that in asthmatic children, ANS parameters correlated positively with severity of their atopic asthma. HRV and HRDB, two easily performed parameters related to the primary PNS dysfunctions correlate to the level of severity of asthma symptoms. We also observed that SSR, which primarily indicates SNS dysfunctions was not correlated with asthma severity in children.

Table 2
Statistical comparison of autonomic nervous system variables between of study and control groups.

Parameter	Group I (mild)	Group II (moderate)	Group III (severe)	Control group	p
SNN50 count (unit)	12,455 ± 5766	16,778 ± 5655	18,500 ± 4521	11,356 ± 6224	0.014
RMSSD (ms)	47 ± 26	73 ± 30	79 ± 35	44 ± 22	0.018
PNN50 (%)	25 ± 4	28 ± 5	32 ± 8	24 ± 6	0.047
HRDB (bpm)	16 ± 3	18 ± 4	21 ± 3	15 ± 2	0.026
HRV-Triangular Index	36 ± 6	35 ± 6	37 ± 7	35 ± 4	0.377
LF (nu)	56 ± 4	53 ± 5	56 ± 5	55 ± 3	0.823
HF (nu)	26 ± 5	34 ± 5	38 ± 4	24 ± 4	0.011
LF/HF	2.1 ± 0.3	1.55 ± 0.53	1.47 ± 0.18	2.3 ± 0.5	0.019
SSR (latency, ms)	0.90 ± 0.4	0.88 ± 0.2	0.93 ± 0.4	1.02 ± 0.3	0.645
Cardiovagal subscore	0.43	0.48	0.52	0.05	0.044
Adrenergic subscore	0.05	0.03	0.04	0.04	0.262
CANSS	1.76	2.32	2.45	0.12	0.023

SNN50 count, number of R–R intervals exceeding 50 ms; RMSSD, root mean square of successive differences between normal sinus R–R intervals; PNN50, the percentage of difference between adjacent normal R–R intervals that are greater than 50 ms computed over the entire height of histogram created by the charting all the RR intervals; HRDB, heart rate to deep breathing; HRV-Triangular index, the ratio of number of all R–R intervals to the all averaged normal sinus R–R, LF, low frequency power; HF, high frequency power; SSR, sympathetic skin response; CANSS, composite autonomic nervous scoring scale

Table 3

Comparison of various ANS function parameters according to the disease severity in children with asthma.

	Group I (mild)	Group II (Moderate)	Group III (Severe)	<i>p</i>
PNN50 (%) mean (95%CI)	24(19, 28)	27(23, 31)	34(30, 38)	0.035
SNN50 count (unit) mean (95%CI)	10(8,14)	14(11, 20)	17(15,23)	0.002
HRDB (bpm) Mean (95%CI)	14(13, 17)	18(16, 23)	22(19,26)	0.020
LF (n.u) mean (95%CI)	49(47, 53)	51(48, 55)	52(48,56)	0.440
HF (n.u) mean (95%CI)	23(20, 29)	30(28, 35)	35(33,41)	0.006
LF/HF median (95%CI)	1.8(1.6, 2.1)	1.4(1.3, 1.7)	0.9(0.8, 1.2)	0.033
SSR (latency, ms) mean (95%CI)	0.8 (0.7, 1.3)	0.9(0.7, 1.4)	0.9(0.8, 1.3)	0.744

SNN50 count, number of R–R intervals exceeding 50 ms; PNN50, the percentage of difference between adjacent normal R–R intervals that are greater than 50 ms computed over the entire height of histogram created by the charting all the RR intervals; HRDB, heart rate to deep breathing; LF, low frequency power; HF, high frequency power; SSR, sympathetic skin response;

Many disorders in humans have been suspected as being related to or caused by ANS abnormalities. Asthma is certainly one such disorder (Goyal et al., 2010). Kallenbach et al. (1985), demonstrated a defined relationship between the magnitude of respiratory sinus arrhythmia and the degree of bronchial hyper-reactivity (BHR), a characteristic of asthma, which may be related to enhanced parasympathetic nervous activity. PNS functions are controlled mainly by nucleus tractus solitarius (NTS) neurons that receive vagal sensory information from cardiorespiratory and subdiaphragmatic organs within the gastrointestinal tract. NTS neurons project directly or indirectly to structures of the medulla that regulate the activity of the sympathetic and parasympathetic preganglionic neurons. The activity of the parasympathetic cardioinhibitory preganglionic neurons localized mainly in the ventrolateral part of nucleus ambiguus (NA) and to a lesser extent in the dorsal motor nucleus of the vagus is increased probably via direct excitatory pathways originating from the NTS (Dergacheva et al., 2010; Ondicova and Mravec, 2010).

Syme (2005) proposed that NTS hypersensitivity could increase BHR leading to worsening of asthma. Stable or steroid-treated asthmatics demonstrated PNS activity, which contradicts the view that higher PNS activity is a secondary contributor to airway inflammation (Du et al., 2001). The parasympathetic innervation mediates both cholinergic contractions and non-adrenergic, non-cholinergic (NANC) relaxations of the airway (Yip et al., 1981). Nitric oxide (NO) and vasoactive intestinal peptide (VIP) have been identified as a major neurotransmitters of i-NANC mediated relaxation and are co-localized in cholinergic nerve endings (Maggi et al., 1995). In humans, NO is a more important mediator, as i-NANC responses were found to be totally locked by L-NAME (N (G)-nitro-L-arginin methyl ester), which is a nitric oxide synthase (NOS) inhibitor (Belvisi et al., 1992). The PNS hyperactivity increases the release of (NO). NO may enhance inflammation by altering the balance T helper Th1 and Th2 cells types leading to the proliferation of Th2 lymphocytes, which putatively produce a cytokine profile that has been associated with exacerbation of asthma (van der Valk et al., 2012; Cabral et al., 2009). The resulting inflamed airways can set up a vicious cycle of increased afferent activity leading to further inflammation, increased BHR and increased PSN activity, resulting in a positive correlation between PSN tone and severity of asthma (Ostrowska-Nawarycz et al., 2006).

The analysis of HRV is considered a valid method to use in assessing ANS functions in normal healthy individuals as well as

in patients with various disorders (Silvetti et al., 2001; Ostrowska-Nawarycz et al., 2006). There were two approaches to measure HRV: time domain and frequency domain analyses. In the time domain analysis, SDNN represents a general measurement of ANS balance, whereas the pNN50 predominantly reflects PNS activity. On the other hand, in the frequency domain analysis, HF is modulated predominantly by the PNS, whereas the LF is under the influence of both the parasympathetic and the sympathetic systems. The LF/HF ratio can be considered the marker for the sympathovagal balance (Stein et al., 1994). There are a few studies about HRV in children with asthma. For example, Kazuma et al., found that HRV is decreased in asthmatics (Kazuma et al., 1997). However, it must be also noted that asthmatic subjects can also present with conflicting ANS lab values. In a study by Shelhamer et al. (1983), all the allergic subjects showed abnormal beta-adrenergic hyporeactivity and cholinergic hypersensitivity, whereas generally speaking, allergic asthma is normally associated with excessive alpha-adrenergic responsiveness. In many studies of adults, but not in children, increased PSN tone has been associated with clinical asthma or the worsening of asthma (Jartti et al., 1998; Shah et al., 1990). In this study, we found a significant increase in HF rather than LF and a decrease LF/HF ratio in children with asthma, suggesting a predominance of PNS. The PNS may therefore be more important for respiratory dysfunctions in asthmatic children than previously considered in relationship to adult asthmatics.

One limitation of our study is the lack of investigation of the effect of appropriate medication on ANS functions in severely impaired pulmonary functions. The ethical approval for this sub-population was outside the approved scope of this study. Further studies are needed to evaluate ANS changes after different treatment options in childhood asthma with different stages of severity. Initial ANS functional status and the effect of the treatment may provide clinicians with useful information to choose an appropriate treatment plan.

In atopic diseases, all tissues except at the sites of allergic reaction have decreased sympathetic tone. Some studies showed that in patients with atopic dermatitis and allergic rhinitis, decreased sympathetic tone may be associated with disease severity (Cicek et al., 2008; Tascilar et al., 2009). In our study, however, the SSR results of asthmatic children were not significantly different from control groups. Therefore, we concluded that SNS activity in the asthmatic children remained unaffected. We used SSR, a noninvasive electrophysiological test, to evaluate the SNS (Gutrecht, 1994; Vetrugno et al., 2003). When all the groups with different symptom severities were compared with the control group, statistically different SSR latency was not found in the upper extremities. Additionally, there was no correlation between asthma severity and SSR results in any of the asthmatic groups. This work demonstrates that SSR latencies are not larger than normal and the amplitude of SSR amplitude is not associated with the severity level of asthma in children.

Table 4

Logistic regression analyses of asthmatic groups according to the composite autonomic nervous scoring scale (CANSS).

	Meaningfulness	Rise	Validity percentage
Group I–II	0.278 ($p > 0.1$)	1.001	57%
Group I–III	0.052 ($p < 0.001$)	1.002	78%
Group II–III	0.071 ($p < 0.001$)	1.002	72.8%

In conclusion, this is the first study that shows positive correlation between the severity of asthma in children and ANS dysfunctions. Even in the normal conditions in which the patient is free of an asthma attack, the ANS functions of asthma sufferers differ from those of normal children. It may be that the ANS dysfunction associated with PNS dysfunction rather than SNS hypoactivity in asthmatic children is related to the severity of their asthma. It is speculated that this dysfunction in PNS activity may be one of the causes contributing to the development of disease severity in children with asthma. We recommend further investigation. The advent of ANS testing laboratories as described in this study will certainly enhance our understanding of asthma.

Acknowledgement

The authors have no financial or personal relationships with other people or organization that could pose a conflict of interest in connection with the present work.

References

- American Thoracic Society, 1995. Standardization of spirometry; 1994 update. *American Journal of Respiratory and Critical Care Medicine* 152, 1107–1136.
- Asher, I., 2008. The International Study of asthma and Allergies in Childhood (ISAAC). *New Zealand Medical Journal* 121, 117–118.
- Belvisi, M.G., Stretton, C.D., Miura, M., Verleden, G.M., Tadjkarimi, S., Yacoub, M.H., Barnes, P.J., 1992. Inhibitory NANC nerves in human tracheal smooth muscle: a quest for the neurotransmitter. *Journal of Applied Physiology* 73, 2505–2510.
- Cabral, A.L., Vollmer, W.M., Barbirotto, R.M., Martins, M.A., 2009. Exhaled nitric oxide as a predictor of exacerbation in children with moderate-to-severe asthma: a prospective, 5-month study. *Annals of Allergy Asthma & Immunology* 103, 206–211.
- Chesrown, S.E., Venugopalan, C.S., Gold, W.M., Drazen, J.M., 1980. In vivo demonstration of nonadrenergic inhibitory innervation of the guinea pig trachea. *Journal of Clinical Investigation* 65, 314–320.
- Cicek, D., Kandi, B., Berilgen, M.S., Bulut, S., Tekatas, A., Dertlioglu, S.B., Ozel, S., Saral, Y., 2008. Does autonomic dysfunction play a role in atopic dermatitis. *British Journal of Dermatology* 159, 834–838.
- Claus, D., Schondorf, R., 1999. In: Deuschl, G., Eisen, E. (Eds.), *Recommendations for the practice of clinical neurophysiology (EEG)* (Suppl. 52). Elsevier, pp. 276–277.
- Dergacheva, O., Griffioen, K.J., Neff, R.A., Mendelowitz, D., 2010. Respiratory modulation of premotor cardiac vagal neurons in the brainstem. *Respiration Physiology and Neurobiology* 174, 102–110.
- Du, J., He, J., Wang, Y., 2001. A study of heart rate variability in asthma. *Zhonghua Jie He Hu Xi Za Zhi* 24, 744–745.
- Ducharme, F.M., Davis, G.M., 1998. Randomized controlled trial of ipratropium bromide and frequent low doses of salbutamol in the management of mild and moderate acute pediatric asthma. *Journal of Pediatrics* 133, 479–485.
- Elie, B., Guiheneuc, P., 1990. Sympathetic skin response: normal results in different experimental conditions. *Electroencephalography and Clinical Neurophysiology* 76, 258–267.
- Goyal, M., Jaseja, H., Verma, N., 2010. Increased parasympathetic tone as the underlying cause of asthma: a hypothesis. *Medical Hypotheses* 74, 661–664.
- Gutrecht, J.A., 1994. Sympathetic skin response. *Journal of Clinical Neurophysiology* 11, 519–524.
- Jartti, T.T., 2001. Asthma, asthma medication and autonomic nervous system dysfunction. *Clinical Physiology* 21, 260–269.
- Jartti, T.T., Kaila, T.J., Tahvanainen, K.U., Kuusela, T.A., Vanto, T.T., Välimäki, I.A., 1998. Altered cardiovascular autonomic regulation after salmeterol treatment in asthmatic children. *Clinical Physiology* 18, 345–353.
- Kallenbach, J.M., Webster, T., Dowdeswell, R., Reinach, S.G., Millar, R.N., Zwi, S., 1985. Reflex heart rate control in asthma. Evidence of parasympathetic overactivity. *Chest* 87, 644–648.
- Kazuma, N., Otsuka, K., Matsuoka, I., Murata, M., 1997. Heart rate variability during 24 hours in asthmatic children. *Chronobiology International* 14, 597–606.
- Lin, R.Y., Pesola, G.R., Bakalchuk, L., Morgan, J.P., Heyl, G.T., Freyberg, C.W., Cataquet, D., Westfal, R.E., 1998. Superiority of ipratropium plus albuterol over albuterol alone in the emergency department management of adult asthma: a randomized clinical trial. *Annals of Emergency Medicine* 31, 208–213.
- Lundgren, R., Söderberg, M., Hörstedt, P., Stenling, R., 1988. Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. *European Respiratory Journal* 1, 883–889.
- Maggi, C.A., Giachetti, A., Dey, R.D., Said, S.I., 1995. Neuropeptides as regulators of airway function: vasoactive intestinal peptide and the tachykinins. *Physiological Reviews* 75, 277–322.
- Meessen, N.E., van der Grinten, C.P., Luijendijk, S.C., Folgering, H.T., 1996. Histamine induced bronchoconstriction and end tidal inspiratory activity in man. *Thorax* 51, 1192–1198.
- Morrison, J.F., Pearson, S.B., 1991. The parasympathetic nervous system and the diurnal variation of lung mechanics in asthma. *Respiratory Medicine* 85, 285–289.
- National Heart, Lung and Blood Institute. National Asthma Education and prevention program. Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma, 2007.
- Ollerenshaw, S.L., Jarvis, D., Sullivan, C.E., Woolcock, A.J., 1991. Substance P immunoreactive nerves in airways from asthmatics and nonasthmatics. *European Respiratory Journal* 4, 673–682.
- Ondicova, K., Mravec, B., 2010. Multilevel interactions between the sympathetic and parasympathetic nervous systems: a minireview. *Endocrine Regulations* 44, 69–75.
- Ostrowska-Nawarycz, L., Wroński, W., Błaszczyk, J., Buczyński, K., Nawarycz, T., 2006. The heart rate variability analysis in youth and children with bronchial asthma. *Pol Merkuri Lekarski* 20, 399–403.
- Polgar, P., Promadhat, V., 1971. Pulmonary testing in children. W. B Saunders, Philadelphia, p. 100–153.
- Santanello, N.C., Barber, B.L., Reiss, T.F., Friedman, B.S., Juniper, E.F., Zhang, J., 1997. Measurement characteristics of two asthma symptom diary scales for use in clinical trials. *European Respiratory Journal* 10, 646–651.
- Shah, P.K., Lakhotia, M., Mehta, S., Jain, S.K., Gupta, G.L., 1990. Clinical dysautonomia in patients with bronchial asthma. Study with seven autonomic function tests. *Chest* 98, 1408–1413.
- Shahani, B.T., Halperin, J.J., Boulu, P., Cohen, J., 1984. Sympathetic skin response—a method of assessing unmyelinated axon dysfunction in peripheral neuropathies. *Journal of Neurology Neurosurgery and Psychiatry* 47, 536–542.
- Shelhamer, J.H., Marom, Z., Kaliner, M., 1983. Abnormal beta-adrenergic responsiveness in allergic subjects. II. The role of selective beta 2-adrenergic hyporeactivity. *Journal of Allergy and Clinical Immunology* 71, 57–61.
- Silvetti, M.S., Drago, F., Ragonese, P., 2001. Heart rate variability in healthy children and adolescents is partially related to age and gender. *International Journal of Cardiology* 81, 169–174.
- Stein, P.K., Bosner, M.S., Kleiger, R.E., Conger, B.M., 1994. Heart rate variability: a measure of cardiac autonomic tone. *American Heart Journal* 127, 1376–1381.
- Syme, P., 2005. Are cardiac syndrome X, irritable bowel syndrome and reflex sympathetic dystrophy examples of lateral medullary ischaemic syndromes. *Medical Hypotheses* 65, 145–148.
- Tascilar, E., Yokusoglu, M., Dundaroz, R., Baysan, O., Ozturk, S., Yozgat, Y., Kilic, A., 2009. Cardiac autonomic imbalance in children with allergic rhinitis. *Tohoku Journal of Experimental Medicine* 219, 187–191.
- van der Valk, R.J., Baraldi, E., Stern, G., Frey, U., de Jongste, J.C., 2012. Daily exhaled nitric oxide measurements and asthma exacerbations in children. *Allergy* 67, 265–271.
- van der Velden, V.H., Hulsmann, A.R., 1999. Autonomic innervation of human airways: structure, function, and pathophysiology in asthma. *Neuroimmunomodulation* 6, 145–159.
- Vetrugno, R., Liguori, R., Cortelli, P., Montagna, P., 2003. Sympathetic skin response: basic mechanisms and clinical applications. *Clinical Autonomic Research* 13, 256–270.
- Yip, P., Palombini, B., Coburn, R.F., 1981. Inhibitory innervation to the guinea pig trachealis muscle. *Journal of Applied Physiology* 50, 374–382.