Original Papers • DOI: 10.2478/pneum-2019-0014 • 68 • 2019 • 68-74

Pneumologia

Prognosing a severe course of asthma in children following the study of endothelial function

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Abstract

English:

Background: *Asthma is a common disease with increasing prevalence in children and adults. The WHO estimates that annually 15 million disability-adjusted life-years are lost, and 250,000 asthma deaths are reported worldwide. Approximately, 500,000 annual hospitalizations are due to asthma (1).*

Aim: *In our study, we aimed to evaluate the endothelial function in children with asthma in remission and the prognosis of severe asthma.*

Materials and methods: *The study involved examination of 91 children, aged 6–17 years, with persistent asthma in the remission period. Indices of endothelial function (soluble vascular molecule of intercellular adhesion-1 [sVCAM-1], concentration of stable metabolites of nitric oxide in blood serum [$NO_2 + NO_3$], thickness of the intima-media complex [IMC] of the common carotid artery (CCA), and endothelium-dependent dilatation of the brachial artery [FMD%]). Statistical analyses were performed with StatSoft STATISTICA Version 8 (Tulsa, OK). To determine the relation between qualitative characteristics, the criterion χ^2 was used, and the procedure of multiple logistic regression analysis was performed.*

Results: *The endothelium parameter levels (FMD% [H = 46.02], IMC [H = 60.75], $NO_2 + NO_3$ [H = 40.82], and sVCAM-1 [H = 76.57, p = 0.0000]) depend on the severity of the disease. The study showed that the factors that should be taken into account in prognosis of the formation of the severe course of asthma include positive family allergic history, serum sVCAM-1 and $NO_2 + NO_3$ levels, and the thickness of IMC CCA.*

Conclusions: *All the children with asthma in the remission period were found to have endothelial dysfunction. The degree of disruption of the function of the endothelium depends on the severity of the course of asthma. An algorithm for predicting the severe course of asthma in children has been developed.*

Keywords

asthma • children • prognosis • endothelial dysfunction.

Prezicerea evoluției severe a astmului la copii prin studiul funcției endoteliale

Rezumat

Romanian:

Astmul este o boală cunoscută, cu o prevalență crescândă atât la copii, cât și la adulți. OMS estimează că anual sunt pierduți 15 milioane de ani de viață ajustați în funcție de handicap și 250 000 de decese cauzate de astm sunt raportate în întreaga lume. Aproximativ 500.000 de spitalizări anuale se datorează astmului (1).

În studiul nostru ne-am propus să evaluăm funcția endotelială la copiii cu astm în remisie și prognosticul astmului sever.

Material și metodă. *Studiul a presupus examinarea a 91 copii cu astm persistent în perioada de remisie, cu vârsta cuprinsă între 6 și 17 ani. Indici ai funcției endoteliale: molecula vasculară solubilă a adeziunii intercelulare-1 (sVCAM-1), concentrația de metaboliți stabili ai oxidului nitric în serul de sânge ($NO_2 + NO_3$), grosimea complexului intima-media (IMC) și dilatarea dependentă de endoteliu a arterei brahiale (FMD%). Analizele statistice au fost efectuate cu StatSoft STATISTICA Versiunea 8 (Tulsa, OK). Pentru a determina relația dintre caracteristicile calitative, a fost utilizat criteriul χ_2 și a fost efectuată procedura de analiză logistică de regresie multiplă.*

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Rezultate. *Nivelul parametrilor endoteliului (FMD% (H = 46.02), IMC (H = 60.75), NO2 + NO3 (H = 40.82) și sVCAM-1 (H = 76.57) sunt influențați de severitatea bolii. Factorii de risc ai astmmului sever sunt istoricul familial de alergii, nivelele serice sVCAM-1 și $NO_2 + NO_3$ și grosimea CCA-ului IMC.*
Concluzii. *Toți copiii cu astm în perioada de remisie au fost diagnosticați cu disfuncție endotelială. Gradul de afectare a funcției endoteliului depinde de severitatea cursului astmului. A fost dezvoltat un algoritm pentru prezicerea cursului sever al astmului la copii.*

Cuvinte-cheie

astm • copii • prognostic • disfuncție endotelială.

Introduction

Across the globe, respiratory allergies affect almost 700 million people, and about 330 million cases are affected by asthma (2–4). The increase in the prevalence of asthma in all age groups, the increase in the number of severe and resistant to the treatment forms of disease, and the "debut" of the disease at an early age explain the interest in this problem of modern medical society (5,6). Untimely diagnosis of asthma leads to significant restrictions in various aspects of human life and can cause disability (7). To prevent the development of severe forms of the disease, there is a need to predict asthma progression.

From the point of view of pathophysiological process, asthma is considered as chronic inflammation of the respiratory tract, regulated by various cellular elements and inflammatory mediators (8). The development of chronic inflammation of the bronchopulmonary system is due in no small part to vascular and endothelial factors (9).

The cascade of inflammatory reactions is determined by the activation of the endothelium, which expresses on its surface molecules of intercellular adhesion (in particular, sVCAM-1), resulting in the processes of migration of effector cells and tissue infiltration and vascular inflammation (10,11). Endothelium damage leads to impairment of its normal function. First of all, the synthesis of NO decreases, and therefore the ability of blood vessels to relax is inhibited (12–14).

Chronic inflammation in asthma is not limited to the respiratory tract but is systemic in nature. Microvascular changes are one of the components of respiratory tract remodeling. Consequently, it can be assumed that the degree of endothelial dysfunction may be associated with the severity of the disease (15,16).

It is believed that thorough determination of vascular and endothelial factors in the development of chronic inflammatory process of the bronchopulmonary system may be one of the perspective directions of further scientific study of the mechanisms of development, formation, and progression of asthma (9).

Thus, in our study, we aimed to evaluate the endothelial function in children with asthma in remission and the prognosis of severe asthma.

Materials and methods

Patients and groups

The study involved examination of 91 children with persistent asthma in the remission period (50 boys and 41 girls), aged 6–17 years. The diagnosis was based on GINA 2017 recommendations. The Asthma Control Questionnaire (ACQ) (17) was also used to determine asthma control. The inclusion criteria were (1) signed informed consent to participate in the study given by the parents and children aged from 12 to 17 years, (2) established diagnosis of persistent asthma, and (3) absence of symptoms of bronchial obstruction for 4 weeks. Exclusion criteria were (1) presence of congenital anomalies of the respiratory system, (2) cystic fibrosis, (3) pneumonia and other acute and chronic inflammatory diseases, and (4) patients who received anti-leukotriene drugs and systemic corticosteroids over the past 4 weeks. Depending on the severity of asthma, children were divided into three groups: Group 1 included patients with mild persistent course of asthma (n = 40), Group 2 included patients with a moderate course (n = 34), and Group 3 included patients with a severe course of the disease (n = 17). The control group included 15 practically healthy children of comparable age and sex. The study involved history taking, evaluation of history, assessment of the general condition of children, and general clinical laboratory and instrumental studies.

The pulmonary function test (PFT) and spirometry was performed using the SpiroCom computerized spirometer "KhAImedica."

Evaluation of endothelial function

The examination was conducted in the morning after 12 h of night sleep. For 48 h before the study, the children did not use coffee, vasoactive agents, and did not engage into physical activity.

Venous blood (2 ml) was taken before meals and centrifuged at 2000 rpm for 10 min. Serum in the lower portion of the test tube was stored at –30°C until the assessment. The collected blood samples were analyzed to determine the serum level of the soluble vascular molecule of intercellular adhesion-1 (sVCAM-1) by immunoassay assay using human sVCAM-1 platinum ELISA (eBioscience, Bender MedSystems GmbH, Austria, BMS232) and also to determine the concentration of stable nitrogen oxide metabolites ($NO_2 + NO_3$) spectrophotometrically using Griess reagent.

The function of the endothelium was evaluated using the ultrasound digital ultrasound diagnostic complex "Ultima PA" using a 10–15 MHz sensor. The examination was carried out in a silent purpose-built room maintained at a constant temperature of 22–24°C in the supine position after 10 min of rest. Endothelium-dependent dilatation of the brachial artery [according to Celermajer et al., (1992)] was determined, and a percentage increase in the diameter of the brachial artery (FMD%) was calculated. FMD% is considered normal if the increase in the diameter of the brachial artery after occlusion is more than 10% of the initial one (18). The ultrasonography was used to study the thickness of the intima-media complex of the common carotid artery (IMC CCA) using the method of Pignoli, 1986 (19).

The protocol of this study was in accordance with the ethical guidelines of the Helsinki Declaration of 1975 and was approved by the Ethics Committee of Kharkiv National Medical University.

The study was performed with minimal psychological distress on the part of patients. Patients and their parents were fully informed about the methods and amount of research.

Statistical analyses were performed with StatSoft STATISTICA Version 8 (Tulsa, OK).

The verification of the distribution according to the Gauss law was carried out with the help of the Shapiro–Wilk test. The arithmetic mean and the standard deviation from the mean (SD) were considered as characteristics of the group for signs with distribution corresponding to the Gauss law. For non-Gaussian distribution, the median (Me), interquartile velocity (Lq, lower quartile; Uq, upper quartile) were determined. Comparing the indices characterized by comparison by more than two points, the Kruskal–Wallis (KW) test was used, and differences were considered probable in the context of the Bonferroni amendment.

Nonparametric Mann–Whitney *U*-criterion (MW) was used to compare two independent samples. The χ^2 criterion was used to determine the relation between qualitative characteristics. The hazard ratio (HR) was reported as the relative risk (RR) with 95% confidence intervals.

The procedure of multiple logistic regression analysis was performed in order to find out what factors should be taken into account in prognosis of the severe course of asthma.

Logistic regression models were fitted using a maximum likelihood estimation and adjusted for all potential and measured confounders including age, sex, duration of the disease, the presence of concomitant allergic disorder, positive family allergic history, recurrent respiratory diseases, duration of asthma, serum Ig level, PFT indices, endothelial function indices (serum levels of sVCAM-1, $NO_2 + NO_3$, IMC CCA thickness, FMD%) in the two groups, and we also performed a sensitivity analysis using propensity score matching.

The quality of the elaborated model was tested using the percent concordant (PC). This figure is equal to the fraction of observations that were correctly reclassified into separate subgroups of the dependent index using the logistic regression equation. The closer this indicator to 100%, the higher the quality of the resulting model is.

Mathematically, the logistic regression model is represented as the dependence of the logarithm of the chance of the onset of the predicted event (logit) on the linear combination of factor variables.

$$P = \frac{1}{1 + e^{-z}},$$

where P is the probability of the predicted event;

$z = a + b_1 * X_1 + b_2 * X_2 + ... + b_n * X_n$;

e, mathematic constant 2.72;

a, model constant;

X_1, X_2, X_n, values of independent variables;

b_1, b_2, b_n, coefficients, the calculation of which is the task of binary logistic regression; and

n, a serial number of the predictor included in the equation.

The construction of the logistic regression model was carried out by the method of stepwise exclusion of prognostic factors with the definition of the minimum set of predictors by the estimation of Nagelkerke square.

Results were considered statistically significant at $p < 0.05$.

Results

There was no significant difference in sex and age in all groups ($p > 0.05$). The longest course of the disease was noted in Group 3 patients ($p_{2-3} = 0.0068$, $p_{1-3} = 0.0028$). Also, these

patients were more frequently found to have concomitant allergic disorders, the positive family allergic history, and recurrent respiratory infections (Table 1). No significant difference was found comparing IgE levels in subjects with different degrees of the disease ($p > 0.05$).

Assessment of family allergic history showed that in the majority (68% [62/91], $p = 0.0000$) of patients with asthma, the closest relatives had allergic diseases.

Evaluation of hereditary allergic diseases showed that the most frequent cases of positive family allergic history was found in children with severe disorders ($p < 0.05$). The study showed a relation between the severity of the disease and positive family allergic history (Table 2). That is, in children, with the presence of this circumstance, the risk of development of a severe course of the disease is 7 times higher.

Group 2 and 3 children were found to have recurrent respiratory diseases more frequently than Group 1 children. The statistical processing showed a relation between the severity of asthma and recurrent respiratory disorders (Table 2) and the risk of severe asthma in these patients was 8.9 times higher.

The study also showed a relation between the severity of asthma in children and associated allergic disorders (Table 2). Children with concomitant allergic disorders were found to have a high RR of developing severe course of asthma (3.5 times higher).

Further statistical processing implied a dispersion analysis of KW statistical characteristics of endothelial function parameters (Table 3). It showed that criterion H for such parameters as FMD% ($H = 46.02$, $p = 0.0000$), IMC CCA thickness ($H = 60.75$, $p = 0.0000$), $NO_2 + NO_3$ ($H = 40.82$, $p = 0.0000$), and sVCAM-1 ($H = 76.57$, $p = 0.0000$) of blood serum was highly significant. This gives the right to assert that the statistical characteristics of the relevant indices of different groups were significantly different, and the levels of these indices depended on the patient's group. The most significant decrease in these levels was observed in Group 3 patients (Table 3), which may indirectly indicate the activity of the inflammatory process in the vessel wall and a significant abnormality in the function of the endothelium in patients with severe asthma.

Table 1. General characteristics of children with persistent BA

	Group 1 ***N* = 40**	**Group 2** ***N* = 34**	**Group 3** ***N* = 17**	***P***
Age, years mean ± SD	10.78 ± 3.62	10.75 ± 2.83	10.94 ± 3.14	$p > 0.05$
Girls/boys	22/18	18/16	7/10	$p > 0.05$
Duration of the disease (years) Median (IQR)	3 (1; 4)	5 (2; 7)	9 (6; 11)	$p_{1-2} = 0.0492$ $p_{2-3} = 0.0068$ $p_{1-3} = 0.0028$
Concomitant allergic disorders	22 (55%)	16 (47%)	14 (82%)	$p_{1-2} = 0.2474$ $p_{2-3} = 0.0293$ $p_{1-3} = 0.0103$
Positive family allergic history	27 (68%)	20 (59%)	16 (94%)	$p_{1-2} = 0.4245$ $p_{2-3} = 0.0127$ $p_{1-3} = 0.0408$
Recurrent respiratory diseases	11 (28%)	21 (62%)	15 (88%)	$p_{1-2} = 0.0340$ $p_{2-3} = 0.0604$ $p_{1-3} = 0.0001$
PFT FEV_1,% FEF_{25},% FEF_{50},% FEF_{75},%	94.0 (85.0; 100.5) 87.0 (80.0; 94.5) 87.5 (81.0; 95.5) 88.5 (82.0; 95.0)	85.5 (80.0; 91.0) 92.0 (86.0; 97.0) 91.0 (85.0; 96.0) 86.0 (81.0; 92.0)	81.0 (79.0; 86.0) 79.0 (70.0; 85.0) 82.0 (77.0; 87.0) 84.0 (79.0; 89.0)	$p > 0.05$
IgE, IU/ml Blood serum Median (IQR)	456.1 (248.0; 698.0)	577.0 (180.1; 812.3)	249.3 (65.7; 440.6)	$p_{1-2} = 0.4575$ $p_{2-3} = 0.0735$ $p_{1-3} = 0.0549$
ACQ	0.14 (0.11–0.19)	0.34 (0.24–0.41)	0.60 (0.44–0.86)	$p_{1-2} = 0.0000$ $p_{2-3} = 0.0000$ $p_{1-3} = 0.0000$

Table 2. Risk factors for severe asthma

Variables	**RR**	**95% CI**	χ^2	***p***
Positive family allergic history	7.1	1.991–51.025	6.078	$p < 0.01$
Recurrent respiratory diseases	8.9	2.249–35.555	11.206	$p < 0.01$
Concomitant allergic diseases	3.5	1.080–11.343	5.425	$p < 0.05$

Table 3. Statistical characteristics of endothelial function in children with BA in the remission period (Me [Lq; Uq])

Indices	Children with BA			Control group (n=15)
	Group 1 (n=40)	Group 2 (n=34)	Group 3 (n=17)	
sVCAM-1, mmol/l	885.42 (800.57;990.47)	1150.43 (990.37;1280.77)	1500.18 (1300.32;1700.25)	730.01 (690.63;790.19)
MW *U* Test: $p_{1\text{-}2}$=0.0000; $p_{1\text{-}3}$= 0.0000; $p_{2\text{-}3}$= 0.0000; $p_{c\text{-}1}$= 0.0000; $p_{c\text{-}2}$= 0.0000; $p_{c\text{-}3}$= 0.0000				
NO_2+NO_3, mcmol/l	35.47 (32.00; 42.01)	28.73 (25.23; 34.48)	26.18 (22.57; 32.42)	41.19 (38.22; 43.23)
MW *U* Test: $p_{1\text{-}2}$=0.0001; $p_{1\text{-}3}$= 0.0000; $p_{2\text{-}3}$= 0.0645; $p_{c\text{-}1}$= 0.0327; $p_{c\text{-}2}$= 0.0000; $p_{c\text{-}3}$= 0.0000				
FMD%	11.27 (8.28; 13.33)	10.12 (8.88; 11.76)	8.33 (6.45; 8.82)	23.33 (17.07; 27.77)
MW *U* Test: $p_{1\text{-}2}$=0.2952; $p_{1\text{-}3}$= 0.0010; $p_{2\text{-}3}$= 0.0005; $p_{c\text{-}1}$= 0.0000; $p_{c\text{-}2}$= 0.0000; $p_{c\text{-}3}$= 0.0000				
IMC CCA thickness, mm	0.8 (0.8; 0.9)	1.0 (0.8; 1.1)	1.1 (1.1; 1.2)	0.6 (0.5; 0.7)
MW *U* Test: $p_{1\text{-}2}$=0.0019; $p_{1\text{-}3}$= 0.0000; $p_{2\text{-}3}$= 0.0000; $p_{c\text{-}1}$= 0.0000; $p_{c\text{-}2}$= 0.0000; $p_{c\text{-}3}$= 0.0000				

Table 4. Statistical characteristics of the multiple logistic regression of factors potentially able to have an impact on the development of severe bronchial asthma in children

	Coefficient	Standard coefficient	p
Constant	0.633	-	
Positive family allergic history	0.359	0.383	<0.001
sVCAM-1	0.00063	0.250	<0.001
IMC CCA thickness	0.477	0.160	<0.001
NO_2+NO_3	0.0147	0.186	<0.001

$R = 0.890$, $R^2 = 0.800$, $F = 65.993$, $p < 0.001$.

The study showed that the factors that should be taken into account during the prediction of the development of severe asthma were related to the positive family allergic history, the level of sVCAM-1 in serum, IMC CCA thickness, and serum $NO_2 + NO_3$ levels (Table 4).

According to the results of the analysis, a multiple regression equation was created:

$$z = 0.633 + (0.359 \times \text{positive family allergic history}) + (0.00063 \times \text{sVCAM-1}) + (0.477 \times \text{IMC CCA thickness}) + (0.0147 \times NO_2 + NO_3).$$

Discussion

We have determined that the severe course of asthma to a significant extent depends on a high level of sensitization, as well as the impact of respiratory infections, which enhance the premorbid biological defect.

Asthma is known to be an allergic disorder (2), and therefore the presence of positive family allergic history and concomitant allergic conditions is also an unfavorable factor in the development and persistence of sensitization of the organism, as reflected in our study. Most of the children, especially those with severe asthma, have these symptoms. The greatest duration of the disease is established in patients with severe asthma, which can confirm the logic of the course of the pathological process. As for atopy, we have not identified a reliable difference in the level of IgE blood serum compared it in different groups. There are also no correlations between IgE levels with the severity of the course of asthma. This may indicate more complex mechanisms for the formation of the pathological process.

Our findings, such as the endothelium abnormalities in children with asthma in the remission period, may indicate significant violations of the functional state of the endothelium of the vessels as early as in the mild course of the disease and also speak in favor of the formation of persistent violations, which remain even beyond the activity of the disease. Thus, it is possible to assume continuation of the inflammatory process in the endothelium.

Inflammation leads to the activation of endothelial cells, which are active participants and regulators of the inflammatory process by expressing intercellular adhesion molecules (11). It further contributes to increased vascular permeability (10,11).

Secondary to activated inflammatory and immune mediators due to cytokine production, the immune system undergoes adhesion of leukocytes to the endothelium, and then their transendothelial migration (20,21). There is hypertrophy and hyperplasia of the endothelium, both due to inflammatory changes and due to oxidative stress, which leads to thickening of intima and media, and consequently to ongoing violations of adequate functional activity of the endothelium. These changes are reflected in the enlargement of the IMC vessels, which is shown in this study.

At this stage, the function of the endothelium is actively studied in various pathological conditions, both in children and in adults (22–24). The regulation of vascular tone is

due to the balanced formation and release of vasodilator and vasoconstrictor substances (25). One of the major vascular tone regulators is nitric oxide, which is continuously formed from L-arginine by endothelial NO-synthetase and has a pronounced vasodilating effect. Moreover, NO exhibits antioxidant and bronchodilator effects, suppresses the aggregation and adhesion of platelets, has antiprotective properties, and participates in endothelial-leukocyte interactions and migration of monocytes (26,27).

Such factors as inflammation, hypoxia, and oxidative stress disrupt the normal functioning of the vascular endothelium. These circumstances imply an imbalance of vasoconstrictive and vasodilating substances. NO synthesis reduces, and therefore the ability of vessels to suppress the relaxation (28,29) is suppressed. This leads to the development of various pathological reactions (30,31). The decrease in the level of vasodilating substances and the deterioration of elastic-vascular properties of blood vessels in children with asthma shown in our study confirms endothelial dysfunction and agrees with other studies.

Persistent changes in the endothelium in the future may contribute to the formation of different pathologies, not only of the lungs but also of other systems, in particular cardiovascular, and lead to the development of severe complications (32–34).

According to our findings, not only inflammation and oxidative stress cause changes in the vascular wall but also processes that occur in the endothelium of the vessels can lead to a deterioration of the course of the inflammatory process, forming a vicious circle.

Conclusions

All the children with asthma were found to have endothelial dysfunction. The degree of endothelial function disruption depends on the severity of asthma. The most significant changes were recorded in children with severe asthma. During the study, factors that should be taken into account when predicting a severe course of bronchial asthma were identified, such as positive family allergic history, serum levels of sVCAM-1 and NO_2 + NO_3, and IMC CCA thickness. With the use of these criteria, an algorithm for the prognosis of asthma is created, which may contribute to early and reliable prediction of the course of bronchial asthma in children.

Acknowledgements

The authors would like to thank the parents for allowing their children to participate in the study. We acknowledge the wholehearted support of the clinicians, nurses, and lab personnel who contributed their efforts and made this study possible.

This work was supported by the Kharkiv National Medical University (No. 0111U001173). The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the paper.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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