Cytowanie: Rakowska-Silska M, Lipińska-Opałka AM, Jobs K, Zieniuk K, Rustecka A, Kalicki B: Alergia jako potencjalna przyczyna zaburzeń mikcji u dzieci.
Pediatr Med Rodz 2023; 19 (4): 343–352. DOI: 10.15557/PiMR.2023.0055.

Magda Rakowska-Silska[1], Agnieszka Maria Lipińska-Opałka[1], Katarzyna Jobs[1], Krystyna Zieniuk[2], Agnieszka Rustecka[1], Bolesław Kalicki[1]

Otrzymano: 19.07.2023
Zaakceptowano: 31.08.2023
Opublikowano: 29.12.2023

Alergia jako potencjalna przyczyna zaburzeń mikcji u dzieci

Allergy as a potential aetiology of micturition disorders in children

[1] Department of Paediatrics, Paediatric Nephrology and Allergology, Military Institute of Medicine – National Research Institute, Warsaw, Poland
[2] Department of Pathomorphology, Military Institute of Medicine – National Research Institute, Warsaw, Poland
Adres do korespondencji: Agnieszka Maria Lipińska-Opałka, Department of Paediatrics, Paediatric Nephrology and Allergology, Military Institute of Medicine – National Research Institute, Szaserów 128, 04-141 Warsaw, Poland, tel.: +48 22 261 817 236, e-mail: alipinska@wim.mil.pl

https://doi.org/10.15557/PiMR.2023.0055

ORCID iDs
1. Magda Rakowska-Silska https://orcid.org/0000-0003-3471-141X
2. Agnieszka Maria Lipińska-Opałka https://orcid.org/0000-0001-9429-107X
3. Katarzyna Jobs https://orcid.org/0000-0002-8616-2347
4. Krystyna Zieniuk https://orcid.org/0000-0002-8770-5497
5. Agnieszka Rustecka https://orcid.org/0000-0003-3012-6942
6. Bolesław Kalicki https://orcid.org/0000-0003-1606-5100

Streszczenie

Wprowadzenie i cel: W populacji pediatrycznej często obserwuje się współwystępowanie zaburzeń mikcji z chorobami alergicznymi. Celem pracy była próba wykazania etiologii alergicznej jako potencjalnej przyczyny zaburzeń mikcji u dzieci. **Materiał i metody:** Do badania włączono 69 dzieci, które podzielono na dwie główne kohorty: pacjentów z zaburzeniami mikcji oraz bez tych zaburzeń. Wśród nich wydzielono podgrupy dzieci z alergią oraz dzieci, u których nie stwierdzono reakcji alergicznych. U wszystkich pacjentów oznaczono bezwzględną i procentową zawartość eozynofilów we krwi, oznaczano stężenie IgE całkowitego oraz obecność przeciwciał swoistych dla alergenów pokarmowych i powietrznopochodnych, ponadto oznaczano wybrane parametry immunologiczne. Oceniono również przydatność małoinwazyjnych metod diagnostycznych w diagnostyce alergicznej etiologii zaburzeń mikcji u dzieci. **Wyniki:** Wykazano, że w kohorcie pacjentów z zaburzeniami mikcji monosymptomatyczne moczenie nocne występowało statystycznie częściej w grupie dzieci z atopią w porównaniu z dziećmi bez atopii. Nie stwierdzono istotnych statystycznie różnic między grupami w zakresie: odsetka limfocytów T regulatorowych; stężenia badanych cytokin; wartości bezwzględnych oraz odsetka eozynofilów we krwi. Nie wykazano przydatności cytologii moczu i wymazu z cewki moczowej w ocenie reakcji alergicznych w obrębie pęcherza moczowego. **Wnioski:** Wyniki przeprowadzonych badań wskazują, że monosymptomatyczne moczenie nocne istotnie częściej współwystępuje z alergią niż inne rodzaje zaburzeń mikcji. Nie potwierdzono istotności badania cytologicznego moczu oraz wymazu z cewki moczowej w kierunku obecności eozynofilów w wykazaniu alergicznej etiologii zaburzeń mikcji u dzieci.

Słowa kluczowe: alergia, moczenie nocne, zaburzenia oddawania moczu, eozynofile, neurotoksyna pochodzenia eozynofilowego

Abstract

Introduction: In the paediatric population, the co-occurrence of micturition disorders with allergic diseases is often observed. The aim of this study was to demonstrate whether allergic aetiology may be a potential cause of micturition disorders in children. **Materials and methods:** The study included 69 children who were divided into two main cohorts: patients with and without voiding disorders. Among them, subgroups of children with allergies and children without allergic reactions were distinguished. In all patients, the absolute and percentage content of eosinophils in the blood and selected immune parameters were assessed. The usefulness of minimally invasive diagnostic methods in the diagnosis of allergic aetiology of micturition disorders in children was also evaluated. **Results:** It was shown that in the cohort of patients with voiding disorders, monosymptomatic nocturnal enuresis occurred statistically more commonly in the group of children with atopy compared to the children without atopy. There were no statistically significant differences between the groups in terms of the percentage values of immune parameters. It was found that urine cytology and urethral swab were not helpful tools in assessing allergic reactions in the bladder. **Conclusions:** The results of the study indicate that monosymptomatic nocturnal enuresis co-occurs with allergy significantly more often than with other types of voiding disorders. The importance of urine cytology and urethral smear for the presence of eosinophils in demonstrating the allergic aetiology of micturition disorders in children was not confirmed.

Keywords: allergy, nocturnal enuresis, voiding disorders, eosinophils, eosinophil-derived neurotoxin

INTRODUCTION

Micturition disorders are among the most common problems encountered in paediatric practice. They can lead to the development of emotional disorders, both in patients themselves and their nearest relatives. Children with voiding disorders, including nocturnal enuresis (bedwetting), have low self-esteem and often avoid contact with their peers, which may further aggravate the problems[1]. Although urinary symptoms are most typically functional in nature, their organic origin should always be ruled out[2]. In approximately 85% of children, voiding disorders manifest as isolated nocturnal enuresis, mainly in the form of monosymptomatic nocturnal enuresis (MNE).

MNE is diagnosed in children over 5 years of age who have no other lower urinary tract symptoms (LUTS). To establish the diagnosis, at least one episode of bedwetting per month for a minimum of 3 months should be present[3]. Non-monosymptomatic nocturnal enuresis (NMNE) – is diagnosed in patients >5 years of age who, in addition to nocturnal incontinence, have other LUTS, e.g. daytime enuresis, urgency, or pollakiuria. This type of bedwetting is less common and requires specialist diagnostics to exclude urinary tract defects and disorders or systemic diseases such as diabetes, pituitary gland disease or chronic kidney disease. Secondary bedwetting can also be associated with a history of psychological trauma, abuse, or mental illness.

The relationship between certain types of micturition disorders and allergic diseases has been observed for many years. There have been many case reports of patients with concurrent exacerbation of symptoms of allergic diseases and enuresis. In a number of cases, after identifying and eliminating the allergen from the diet or the environment, symptoms from the respiratory tract and urinary tract disappeared simultaneously[4]. Some studies have shown elevated levels of specific IgE in patients with MNE compared to healthy controls. Boys with nocturnal enuresis were found to have a higher incidence of hay fever, urticaria, food allergies and drug allergies[5,6], while children with bronchial asthma and allergic rhinitis (AR) had a higher incidence of nocturnal enuresis[7–9].

Two diseases with a probable allergic origin and changes in the bladder mucosa have been described so far: interstitial cystitis and eosinophilic cystitis. The aetiology of these conditions has not yet been clearly explained. Studies have shown frequent coexistence of allergy symptoms and LUTS, especially in the case of interstitial cystitis.

The pathophysiological basis of the observed relationship between allergy and LUTS has not been fully elucidated yet. It has been shown that under the influence of irritating factors acting on the bladder wall, chemicals such as adenosine 5'-triphosphate (ATP), acetylcholine, prostaglandins, and nitric oxide are released in the mucous membrane, modulating the activity of nerves and bladder muscles. The bladder mucosa is thought to form a dynamic "sensory structure". Reactions occurring in the bladder may be the cause of symptoms in patients with overactive bladder (OAB) and interstitial cystitis[10]. It was also shown that histamine and other mast cell mediators induced bladder inflammation and hypersensitivity[11]. An increased amount of histamine receptors in the bladder wall was found in patients with interstitial cystitis[12].

The aim of this study was to determine whether allergic aetiology was a potential cause of micturition disorders in children. Disturbances in the immune system (regulatory T cells – Treg lymphocytes, IL-4, IL-5, IL-10) in patients with allergies were assessed, and the relationship between atopy and various types of micturition disorders was evaluated. The usefulness of minimally invasive diagnostic methods (urethral smear test and cytological urine examination) in the diagnosis of allergic aetiology of micturition disorders in children was evaluated. The usefulness of determining the urine concentration of eosinophil-derived neurotoxin (EDN) protein in patients with micturition disorders with a possible allergic origin was assessed as well.

MATERIALS

A total of 69 children (30 boys, 39 girls) aged 5–18 (mean age: 9 years, median: 9 years) hospitalised at the Department of Paediatrics, Paediatric Nephrology and Allergology of the Military Institute of Medicine – National Research Institute in 2018–2021 were included in the study.

The study was conducted in two main cohorts: patients with and without voiding disorders. Among them, subgroups of children with atopy and children without allergic reactions in the IgE-mediated mechanism were distinguished (Tab. 1). The characteristics of the groups are presented in Tab. 2.

The exclusion criteria included congenital defects, chronic diseases and infections of the urinary tract. In addition, patients taking systemic antihistamines (up to 14 days prior to testing) and glucocorticosteroids (up to 3 months prior

With voiding disorders (45)		Without voiding disorders (24)	
Group 1 without atopy (21)	Group 2 with atopy (24)	Group 3 without atopy (8)	Group 4 with atopy (16)

Tab. 1. Division into groups of patients qualified for the study. Number of patients in respective groups given in brackets

Group	Number	Mean age	Minimal age	Maximum age	Gender distribution
1	21	8 years 10 months	5	13	G – 14 (67%) B – 7 (33%)
2	24	8 years 6 months	5	16	G – 14 (58%) B – 10 (42%)
3	8	10 years 11 months	7	15	G – 4 (50%) B – 4 (50%)
4	16	9 years 2 months	5	14	G – 7 (44%) B – 9 (56%)
G – girls; **B** – boys.					

Tab. 2. Characteristics of the groups

DOI: 10.15557/PiMR.2023.0055

Inclusion criteria	Exclusion criteria
Aged 5–18	Urinary tract malformations
Written consent to participate in the study	Chronic diseases of urinary tract
Patient's cooperation	Organic cause of urination disorders
	Urinary tract infection in the last 3 months
	Taking medications that potentially interfere with the interpretation of test results
	Parasitic infection

Tab. 3. Study inclusion and exclusion criteria

to testing) as well as patients with parasitic infections were excluded from the study due to a possible impact on test results. A prerequisite for participating in the study was the written consent of children's legal guardians and patients over 16 years of age. The inclusion and exclusion criteria are presented in Tab. 3.

METHODS

The groups of children with and without voiding disorders were distinguished based on interviews and physical examinations with a thorough assessment of the occurrence and type of voiding disorders. If the patient's parents or the patient reported LUTS, the diagnostic work-up was extended to exclude an organic cause of symptoms. In patients with specific indications, an urodynamic test and voiding cystourethrogram were performed. The presence of underlying organic causes of disorders excluded patients from the study.

The cohort of patients with voiding disorders included children with such LUTS:

- nocturnal enuresis (including MNE);
- daytime incontinence;
- urgency (defined as an uncontrollable urge to urinate);
- pollakiuria (defined as >7 micturitions per day);
- dysuric symptoms (discomfort when urinating).

Serum total and specific IgE levels were determined, and skin prick tests were performed to divide patients into the subgroups with or without atopy. The group of children with atopy consisted of patients with elevated total IgE levels (after exclusion of other causes of the condition) and/or elevated levels of specific IgE towards at least one allergen in class higher than 1. Skin tests were helpful in patients with borderline IgE concentrations. The following food allergen extracts were used: apple, cocoa, orange, banana, peanut, hazelnut, wheat flour, cow's milk, hen's egg white, hen's egg yolk, cod, tomato; and the following extracts of airborne allergens: rye, birch, alder, hazel, mugwort, plantain, lanceolate, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, dog, cat, *Alternaria alternata*, *Cladosporium*.

The absolute and percentage levels of eosinophils in the blood, selected immune parameters including the percentage content of natural regulatory T cells ($CD4^+/CD25^{high}/CD127^{low}/FoxP3^+$), the concentration of IL-4, IL-5, IL-10, the concentration of EDN protein in the morning urine sample were determined, and the percentage of eosinophils in the urethral swab and urine tests was assessed.

The concentration of IL-5 in the serum was determined using an immunoenzymatic method (DuoSet® ELISA test, R&D systems) at the Military Institute of Hygiene and Epidemiology in Warsaw, according to the procedure provided by the manufacturer.

The concentrations of IL-4, IL10 and nTreg cytokines were analysed with a cytometric method (FACSCalibur Flow Cytometer, USA). nTreg cells expressed $CD4^+/CD25^{high}$, $CD4^+/CD25^{high}/CD127^{low}$ and $CD4^+/CD25^{high}/CD127^{low}/FoxP3^+$.

Cytological examinations for eosinophils were carried out in the Laboratory of Molecular Genetics of the Department of Pathomorphology of the Military Institute of Medicine – National Research Institute. Slides were stained with haematoxylin and eosin, as well as with the May–Grünwald–Giemsa method.

EDN protein concentration in urine was determined using an immunoenzymatic method (DuoSet® ELISA test, MBL Medical and Biological Laboratories CO LTD) at the Military Institute of Hygiene and Epidemiology in Warsaw, according to the procedure provided by the manufacturer.

The test results were subjected to statistical analysis. Calculations were performed using the R program, version 3.6.2, together with packages. Throughout the analysis, $p < 0.05$ was considered significant. The hypothesis of normal distribution was assessed with the Shapiro–Wilk test. For the variables, whose distribution met the assumptions of the Gaussian distribution, the mean as the central measure and the standard deviation as the measure of the dispersion were used. Median and quartiles were used for the variables whose distributions differed significantly from the Gaussian distribution. To compare the differences between the groups, for the variables meeting the criteria of Gaussian distribution, parametric tests were used: Student's *t*-test for comparison of the two groups and the ANOVA test for a comparison of more than two groups. For the non-Gaussian variables, non-parametric tests were used: Wilcoxon's test to compare the medians of the two groups and the Kruskal–Wallis test when comparing more than two groups. In the assessment of the percentage variables, the proportion test was used.

RESULTS

Groups of children with and without atopy were compared in terms of the type of micturition disorders. In the cohort of patients with voiding disorders, MNE was found to occur statistically more often in the group of children with atopy compared to the children without atopy ($p = 0.02$). In both groups, other types of micturition disorders dominated (such as NMNE, daytime incontinence, and

 DOI: 10.15557/PiMR.2023.0055

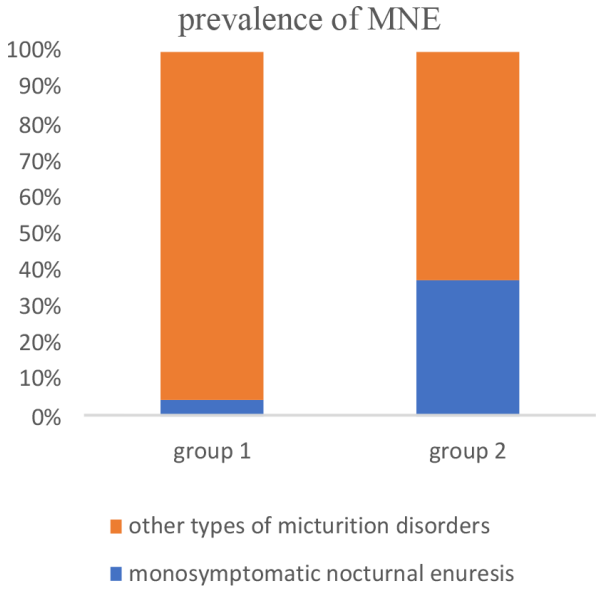

Fig. 1. Comparison of prevalence of MNE in allergic patients (group 2) and non-allergic patients (group 1)

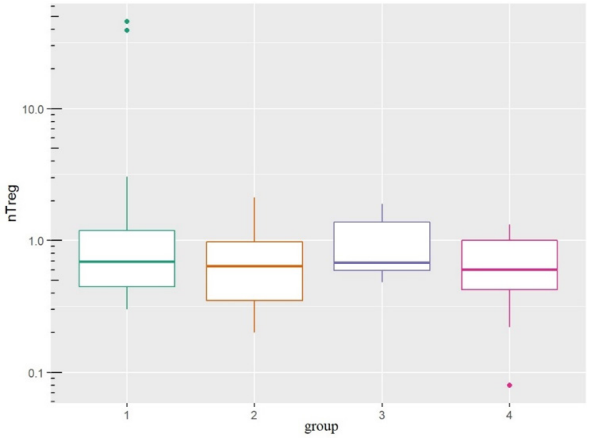

Fig. 2. Percentages of nTreg cells in groups on a logarithmic scale

overactive bladder). In the group of children without allergies, they occurred in 95.2% of patients (20/21), and in the group with allergies – in 62.5% of patients (15/24) (Fig. 1).

The study analysed the immune parameters involved in the mechanism of the allergic reaction. In all groups of patients, the serum parameters such as Treg lymphocytes, IL-4, IL-5, and IL-10 were determined. However, there were no statistically significant differences between the groups in terms of the percentage of Treg lymphocytes (Tab. 4, Fig. 2) or the concentration of IL-5. In the case of IL-10, its lowest level was found in the group of patients with atopy and concomitant voiding disorders, and in the case of IL-4, the highest concentration was found in patients with atopy but without voiding disorders (Tab. 5, Figs. 3–5). However, both results were statistically insignificant ($p = 0.1153$ and $p = 0.1153$, respectively).

It was determined whether the concentration of eosinophils in the blood or their presence in the urine and urethral swab tests differed in patients in the study groups. There were no statistically significant differences between the groups in the absolute values of blood eosinophils ($p = 0.274$) and in the percentage of blood eosinophils ($p = 0.27$) (Tab. 6).

It was shown that urine cytology combined with the assessment of the presence of eosinophils was not a helpful tool in determining the allergic reactions in the bladder. The vast majority of children had no eosinophils in urine (74%, 51/69). Furthermore, there were no statistically significant differences between the study groups ($p = 0.8605$). Similar results were obtained when analysing the presence of eosinophils in the urethral swab test – they were not detected in 84% (57/68) of patients and the results did not differ between the groups.

Analysing whether the cause of LUTS may be an allergic reaction, the concentration of EDN protein in the urine was assessed.

A statistically significant higher concentration of EDN protein in the urine was found in the patients with voiding disorders compared to the patients without such symptoms – $p = 0.0458$. However, this difference became statistically insignificant after conversion to creatinine concentration (Tab. 7).

DISCUSSION

In the Department of Paediatrics, Paediatric Nephrology and Allergology of the Military Institute of Medicine – National Research Institute, the coexistence of atopic

Parameter	Group 1	Group 2	Group 3	Group 4	*p*
Percentage of Treg lymphocytes, median (q25–q75)	0.69 (0.445–1.19)	0.64 (0.35–0.98)	0.68 (0.592–1.38)	0.61 (0.425–1.01)	$p = 0.5022$
q25 – lower quartile; **q75** – upper quartile.					

Tab. 4. Percentages of Treg lymphocytes in the study groups

Parameter	Group 1	Group 2	Group 3	Group 4
IL-4, median (q25–q75)	2.26 (1.42–3.05)	2.03 (1.11–3.36)	3.23 (2.56–3.60)	3.58 (2.30–4.29)
IL-5, median (q25–q75)	1.88 (1.37–2.41)	1.67 (1.37–2.03)	1.40 (0.396–2.52)	2.28 (1.29–3.60)
IL-10, median (q25–q75)	2.17 (1.63–3.20)	1.62 (1.05–2.42)	2.92 (1.41–3.98)	2.93 (1.92–4.26)
q25 – lower quartile; **q75** – upper quartile.				

Tab. 5. IL-4, IL-5, IL-10 serum concentrations in the study groups

DOI: 10.15557/PiMR.2023.0055

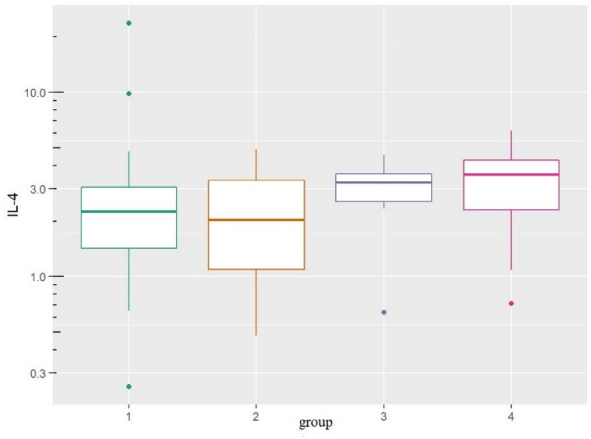

Fig. 3. IL-4 concentrations in groups on a logarithmic scale

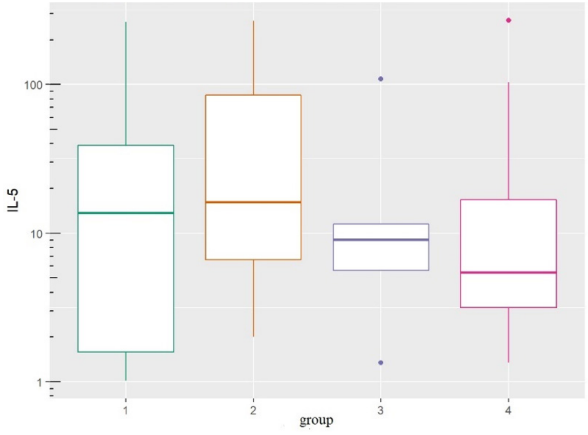

Fig. 4. IL-5 concentrations in groups on a logarithmic scale

diseases with voiding disorders is often observed. In addition, it has frequently been noted that proper treatment of allergic diseases results in the reduction of symptoms from the urinary tract. Therefore, the aim of this study was to determine whether in a certain group of patients with micturition disorders, allergic reactions occurring in the bladder mucosa may be the cause of LUTS.

There are not many reports in the literature on the relationship between micturition disorders and allergic diseases. Most of the studies and case reports relate to eosinophilic

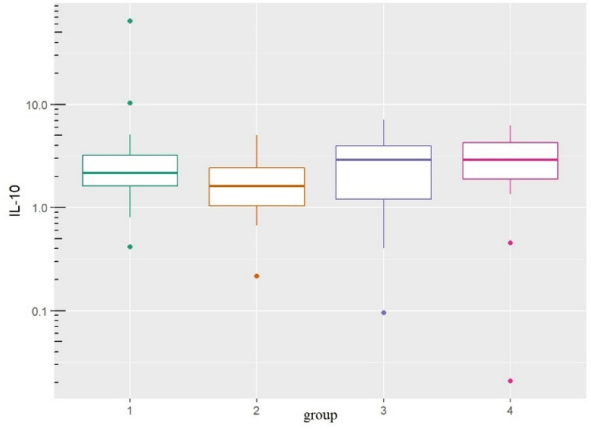

Fig. 5. IL-10 concentrations in groups on a logarithmic scale

cystitis and interstitial cystitis. In these two diseases, allergic reactions have been revealed as one of potential pathomechanisms[13,14]. Eosinophilic cystitis is a very rare disease. To establish a diagnosis, a histological examination of a bladder mucosal sample is required, which is an invasive procedure[15]. For the purpose of the present study, the selection of patients was not limited to individuals with a suspicion of eosinophilic cystitis. The study consisted of a wider group of children with concomitant allergies and micturition disorders.

One of the few available studies on the coexistence of atopic diseases and voiding disorders, conducted by Spanish authors, describes a possible allergic aetiology of urinary symptoms. It has been suggested that allergic reactions may occur in the urinary bladder as well as in the nasal mucosa, in the bronchi, and in the skin[16]. Some allergens have been found to reduce the functional capacity of the bladder and cause detrusor instability[16,17]. Moreover, Zaleski et al. observed a higher incidence of AR, urticaria and allergic reactions to food and drugs in boys with bedwetting[5]. Ozkaya et al. showed a higher incidence of MNE in patients with bronchial asthma (26%) compared to the control group (11%). Moreover, bedwetting was observed more frequently in patients with uncontrolled asthma than in children with well-controlled asthma, as well as in patients with coexisting AR and asthma[9]. Similar conclusions were drawn

Parameter	Group 1	Group 2	Group 3	Group 4
Eosinophils [$\times 10^3$/μL], median (q25–q75)	0.2 (0.13–0.24)	0.235 (0.118–0.492)	0.18 (0.138–0.275)	0.25 (0.17–0.503)
Eosinophils [%], median (q25–q75)	3 (2–4.3)	3.9 (2.18–6.72)	3.15 (2.75–4.05)	3.55 (2.63–8.62)
q25 – lower quartile; **q75** – upper quartile.				

Tab. 6. Concentrations of eosinophils in the study groups

Parameter	Group 1	Group 2	Group 4
EDN [ng/mL], arithmetic average ± *SD*	30.2 ± 15.1	36.8 ± 14.3	16.0 ± 3.98
EDN/creatine, median (q25–q75)	0.238 (0.182–0.522)	0.313 (0.185–0.435)	0.360 (0.304–0.368)
SD – standard deviation; **q25** – lower quartile; **q75** – upper quartile.			

Tab. 7. Urine EDN protein concentrations in the study groups

 DOI: 10.15557/PiMR.2023.0055

by Tsai et al. in their study. The authors confirmed that the risk of enuresis increased with the number of co-occurring allergic diseases[18].

In the presented study, the prevalence of allergic diseases in children with micturition disorders and the prevalence of micturition disorders in children with allergic diseases were not assessed. However, it was shown that among patients with voiding disorders, MNE occurred statistically significantly more frequently in the group of allergic children compared to non-allergic children. In both groups, however, other types of micturition disorders dominated (NMNE, daytime enuresis, overactive bladder detrusor). Higher incidence of other types of micturition disorders probably stems from the fact that children qualified for the study had indications for hospitalisation, while in the case of MNE most patients remain under outpatient care.

In patients with allergies, instability of the bladder detrusor muscle and reduction of the functional volume of the bladder being a result of the allergic reaction should be symptomatic both during the day and at night. However, according to the observations reported in the study by Mungan et al., some patients ignore symptoms occurring during the day and often fail to report them to the doctor[19].

In recent years, allergy researchers have focused mainly on the immune aetiology of atopic diseases. One of the immune mechanisms leading to the development of allergic diseases is imbalance of Th1/Th2 lymphocytes. Another direction of research, aimed at exploring the immunological basis of the hygiene theory, is the assessment of the impact of Th17 lymphocytes and natural Treg lymphocytes on the occurrence of allergic reactions. It has been suggested that their dysfunction or reduced number may adversely affect the process of building tolerance to allergens[20]. Weaker stimulation of immunity in childhood by microbiological factors, resulting from an improvement in the standard of living, leads to a weaker induction of Treg cells[21]. The above-mentioned theory is confirmed by the study results obtained by many researchers who have observed that a decrease in the average percentage of nTreg lymphocytes is inversely correlated with the incidence of atopic diseases. One example is the study by Shi et al., which revealed a statistically significantly lower percentage of $CD25^+FoxP3^+$ lymphocytes among $CD4^+$ cells in patients with bronchial asthma. In addition, the lower the level of nTreg lymphocytes in the blood of patients, the more severe the course of the disease was observed[22]. The same findings were made in some studies on atopic dermatitis[23–26]. One study showed a reduced level of nTreg in allergic patients, especially with chronic exposure to the allergen[27]. A possible defect in the regulatory functions of $CD4^+CD25^+$ lymphocytes is also emphasised, whereby despite a correct or increased number, they are not able to inhibit activated $CD4^+CD25^-$ lymphocytes in patients with AR[28].

However, there are also studies in which contradictory results were obtained, with no differences in the percentage of nTreg lymphocytes found between patients with allergic diseases and healthy people[29,30]. These discrepancies may be attributed to difficulties in the selection and identification of Treg markers[26].

Similarly, in this study, no significant differences were found between the groups of children with and without allergies in terms of the percentage of Treg lymphocytes. As nTreg lymphocytes with a high expression of CD25 (IL-2 α chain) and a low expression of CD127 (IL-7 receptor) were assumed, which is consistent with the data from the literature[31]. Wawrzyniak et al. showed that Treg percentages depended on the time of the year in which the tests were performed: they were higher in the spring-summer period than in the autumn-winter period[32]. This variable, however, was not included in the current study, which may have potentially affected the results.

In the present study, no relationship was found between the occurrence of micturition disorders in children and the number of Treg lymphocytes. No other studies addressing this issue have been found in the available literature.

Another important direction in the assessment of immune reactions in the context of atopic diseases is the determination of the levels of interleukins directly involved in the allergic process. These are mainly IL-4, IL-5, IL-10.

IL-4 and IL-13 are the key cytokines in immune reactions in the Th2 mechanism, and thus represent an extremely important factor in the development of allergies[33–35]. It has been repeatedly shown that in patients with atopy the process of regulating the production of IL-4 is disturbed. Elevated levels of IL-4 were observed in the serum and bronchial lavage fluids of allergic individuals[36,37]. Moreover, an increased production of IL-4 was found in patients with atopic asthma in response to contact with a mite antigen[37,38]. Both the mechanism of action of IL-4 and its role in allergic reactions are well documented, therefore today the main direction of researchers' interests is to use this knowledge for therapeutic purposes[38,39].

IL-5 plays a central role in the activation, proliferation, degranulation, and migration of eosinophils, which is why it is involved mainly in the late phase of the allergic reaction[40,41]. Reports of many researchers highlight the relationship between high concentrations of IL-5 in serum and in bronchial lavage fluids and the severity of allergic diseases, mainly asthma[42,43]. Zangrilli et al. showed an increased concentration of IL-5 in patients with asthma with a late phase of response to an antigen, in contrast to patients with an early phase of allergic reaction, in whom the value of IL-5 was significantly lower[44]. An increase in the level of IL-5 during asthma exacerbations was also observed[45].

Another cytokine with an extremely important role in the mechanism of allergic reaction, is IL-10. It has strong anti-inflammatory and anti-allergic effects, inhibiting the synthesis of pro-inflammatory cytokines, such as interferon γ, IL-2, IL-3, TNF-α or granulocyte-macrophage colony-stimulating factor (GM-CSF). Recently, its pleiotropic effect has also been highlighted. In some diseases, IL-10 promotes the development of an allergic reaction[46]. As early as in the

DOI: 10.15557/PiMR.2023.0055

1990s, decreased levels of IL-10 in asthmatic patients were observed in the bronchial lavage fluid[47,48]. Mouse models demonstrated a markedly enhanced immune response to many allergens and significant eosinophil infiltration in the airways of individuals devoid of IL-10[49]. Lim et al. observed that the gene polymorphism of this cytokine leading to a decrease in its concentration determines the development and severity of allergic reactions within the respiratory tract in humans[50]. These studies highlight the importance of IL-10 in controlling the course of allergic reactions. In our study, no statistically significant differences were found between the groups in terms of IL-4, IL-5, and IL-10 concentrations. These results, different from most data reported in the literature, may be explained by the heterogeneity of the study group in terms of the severity of allergic reactions. Patients with a documented hypersensitivity reaction were assigned to the group of children with atopy, which in many cases is not synonymous with the presence of symptoms at the time of testing. Researchers observed particularly significant deviations in the levels of the discussed cytokines during exacerbations of allergic diseases.

However, despite the lack of statistical significance, the lowest concentration of IL-10 was found in the group of patients with atopy and micturition disorders, while the highest concentration of IL-4 was found in the group of patients with atopy without micturition disorders, which is consistent with the knowledge about the involvement of these cytokines in the mechanism of allergic reaction.

In the reports of many researchers, the topic of cytological examination for the presence of eosinophils is discussed as a diagnostic method that can confirm the allergic aetiology of a disease.

For example, multiple attempts have been made to differentiate the causes of conjunctivitis, assuming that if eosinophils are found in the conjunctival swab test, an allergic aetiology of the disease can be confirmed. However, Kari and Haahtela failed to demonstrate a clear relationship between allergic aetiology and the presence of eosinophilic cells in the swabs from the conjunctival sac. The authors summarised the study with the statement that in a significant proportion of patients with conjunctivitis of various aetiology, eosinophils can be found in the smear, but the test does not clearly determine the diagnosis of the disease[51]. In another study conducted by the same researchers, higher eosinophilic cell counts in the swabs from the conjunctival sac were found in patients with inhalant allergy compared to healthy controls. However, as in the previous work, the eosinophil smear was not useful in differentiating the cause of symptoms[52]. In view of the frequently observed increased percentages of eosinophilic cells in the conjunctival swabs without other accompanying allergic symptoms and without signs of atopy in blood tests and skin prick tests, a separate disease entity, called nonallergic eosinophilic conjunctivitis (NAEC), was recognised[53].

Many authors emphasise the usefulness of the nasal swab in the differential diagnosis of AR. Mierzejewska et al. showed a significantly higher prevalence of eosinophils in the nasal swab in the group of children with AR and bronchial asthma compared to the control group. No such tendency was observed in the group of children with atopic dermatitis. The sensitivity of this diagnostic method was estimated at 52.2% in AR, while the specificity of the test in atopic diseases was 94.1%[54]. According to other authors, a nasal swab for eosinophils is not sensitive and specific enough to be used as a basis for diagnosis[55]. Significant results were also obtained by Gelardi et al. The authors showed that in patients with seasonal AR, the cytological smear obtained from a nasal swab differed significantly depending on whether it was collected during or outside the pollen season[56].

Theoretically, testing for eosinophils in the urine sediment could also be a useful diagnostic tool in some cases. There are only scarce studies in the literature evaluating the presence of eosinophils in the urine in various diseases. Acute interstitial nephritis (AIN) is the disease most commonly associated with the presence of eosinophilic cells in the patients' urine. While it is true that eosinophiluria can be seen in patients with AIN, its presence is not sensitive or specific enough to make a diagnosis. Muriithi et al. estimated the sensitivity of the cytological test for the presence of eosinophils in the urine at 31%, and the specificity at 68%[57]. It has also been shown that eosinophiluria is associated with many other diseases of the urinary system. Examples include cystitis, rapidly progressing glomerulonephritis and bladder cancer, but also prostatitis[58]. Another entity associated with the presence of eosinophils in the urine is eosinophilic cystitis, although in practice eosinophils are occasionally found in the urine sediment. A characteristic feature of this disease is the infiltration of eosinophils within the lamina propria of the bladder mucosae and within the muscularis mucosae, but it rarely influences the results of urine cytology[15].

Looking at the available literature, no papers exploring the association between atopic diseases, micturition disorders, and the presence of eosinophils in urine cytology or urethral swabs can be found. Urethral swab tests are performed mainly in adult patients in the microbiological diagnosis of sexually transmitted diseases (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*)[59,60].

In this study, the association was assessed. However, the usefulness of these tests in the diagnosis of micturition disorders was not established. Also, it was observed that the presence of eosinophils in the urine or smear did not result from coexisting atopic diseases in the studied patients.

In recent years, much attention has been paid to eosinophilic proteins, which are markers of the immune reaction mediated by eosinophils. Researchers tend to focus on two of them: EDN (otherwise known as the EPX protein) and ECP (eosinophil cationic protein). The name EDN is due to the toxic effect on the nervous tissue after experimental injection of this protein into the central nervous system of some animals. EDN has the same biochemical, antiviral,

 DOI: 10.15557/PiMR.2023.0055

and neurotoxic properties as ECP. However, unlike ECP, EDN has low cytotoxicity and its concentration in urine may be an alternative to blood serum testing. The most important functions of this protein include toxic antiparasitic activity, inhibition of T lymphocyte proliferation, and ribonuclease activity[61].

Koller et al. showed higher concentrations of these proteins in blood serum, urine, and nasal lavage of patients with asthma compared to the healthy control group[62]. These results were also confirmed in the meta-analysis conducted by Klonoff-Cohen and Polavarapu in 2016, which focused on the concentration of EDN in urine[63]. It was also found that the level of ECP in patients with asthma increases after contact with an allergen, but before the onset of symptoms, and decreases after the allergen has been eliminated from the patient's environment[64]. In the literature, one can also find studies revealing elevated levels of EDN protein in the serum and urine of patients with atopic dermatitis and AR. Moreover, as in the case of bronchial asthma, the severity of symptoms was positively correlated with its concentration[65]. It is recognised that elevated concentrations of the EDN protein in the serum is not due to its penetration into the blood from organs affected by an allergic reaction. It has been shown that in patients with asthma, eosinophils are also activated in the serum and there they secrete toxic mediators which are later excreted in urine. Their concentration in the urine reflects the concentration in the blood serum[62]. In the studies conducted by Nuijsink et al., there was no clear relationship between the amount of eosinophils in the tested material and the concentration of eosinophil proteins. This is probably due to the fact that EDN and ECP provide information on eosinophil activation rather than on eosinophil counts[66]. Similar conclusions were drawn by Chen et al. It was shown that in patients with AR, the serum concentrations of EDN and ECP increase with rising numbers of activated eosinophils[67].

Another important issue is the increased level of EDN protein in patients with interstitial cystitis. In a study conducted by Bouchelouche et al., it was observed that toxic mediators of eosinophils might be involved in the pathogenesis of this disease[68]. Storm van's Gravesande et al. made another interesting observation. They found significant circadian fluctuations in the concentration of EDN protein in the urine, with the highest level observed in the morning[69]. The study by Bouchelouche suggested that this might indicate local production of the protein as a result of activation of eosinophils in the urinary system.

No studies evaluating relationships between the concentration of eosinophilic proteins in urine and urinary tract symptoms have been found in the literature. As outlined above, most publications focus on allergic respiratory diseases and atopic dermatitis. In such cases, the concentration of EDN protein in urine is taken as an exponent of its concentration in serum. In this paper, the concentration of EDN protein in urine was assessed, allowing for the possibility of its local production within the urinary tract. Higher concentrations of the protein were found in patients with micturition disorders (both allergic and non-allergic) compared to patients with allergy, but without urinary tract symptoms. However, these results turned out to be statistically insignificant when converted to creatinine concentration in the tested portion of urine. It remains debatable whether its concentration needs to be converted to creatinine, when considering the secretion of EDN by activated eosinophils in the bladder.

To sum up, for many years attempts have been made to identify the relationship between voiding disorders and atopic diseases. So far, however, it has not been possible to clearly confirm by laboratory tests that some types of allergic reaction may cause specific LUTS. Similarly, in this study, no such relationship was found. It seems that the topic requires further research, involving much larger groups of patients and the use of other markers, e.g. determination of leukotriene concentrations in urine. Perhaps the histopathological evaluation of lesions in the bladder wall could also contribute to verifying the hypothesis put forward in this paper.

Conflict of interest

The authors report no financial or personal relationships with other individuals or organisations that could adversely affect the content of the publication and claim ownership of this publication.

Funding/Support and role of the sponsor

The research was financed by a grant implemented as part of the statutory activity of the Military Institute of Medicine No. 487.

Author contributions

Original concept of study: MR, KJ, BK. Collection, recording and/or compilation of data: MR, KZ. Analysis and interpretation of data: AMLO, MR, KJ, KZ, AR, BK. Writing of manuscript: AMLO, MR. Critical review of manuscript: AMLO, KJ, AR, BK. Final approval of manuscript: AMLO, MR, KJ, KZ, AR, BK.

Piśmiennictwo

1. Skobejko-Włodarska L: [Nocturnal enuresis. Part I – monosymptomatic nocturnal enuresis (MNE)]. Stand Med Pediatr 2016; 13: 100–110.
2. Kuwertz-Bröking E, von Gontard A: Clinical management of nocturnal enuresis. Pediatr Nephrol 2018; 33: 1145–1154.
3. Caldwell PHY, Deshpande AV, Von Gontard A: Management of nocturnal enuresis. BMJ 2013; 347: f6259.
4. Bray GW: Enuresis of allergic origin. Arch Dis Child 1931; 6: 251–253.
5. Zaleski A, Shokeir MK, Gerrard JW: Enuresis: familial incidence and relationship to allergic disorders. Can Med Assoc J 1972; 106: 30–31.
6. Yılmaz-Durmuş S, Alaygut D, Soylu A et al.: The association between monosymptomatic enuresis and allergic diseases in children. Turk J Pediatr 2018; 60: 415–420.
7. Dahan P, de Bessa J Jr, de Oliveira DM et al.: Association between asthma and primary nocturnal enuresis in children. J Urol 2016; 195: 1221–1226.
8. Lai PH, Yang PS, Lai WY et al.: Allergic rhinitis and the associated risk of nocturnal enuresis in children: a population-based cohort study. Int Forum Allergy Rhinol 2018; 8: 1260–1266.
9. Ozkaya E, Aydın SC, Yazıcı M et al.: Enuresis nocturna in children with asthma: prevalence and associated risk factors. Ital J Pediatr 2016; 42: 59.
10. Fry CH, Vahabi B: The role of the mucosa in normal and abnormal bladder function. Basic Clin Pharmacol Toxicol 2016; 119 Suppl 3: 57–62.
11. Grundy L, Caldwell A, Garcia Caraballo S et al.: Histamine induces peripheral and central hypersensitivity to bladder distension via the histamine H_1 receptor and TRPV1. Am J Physiol Renal Physiol 2020; 318: F298–F314.
12. Shan H, Zhang EW, Zhang P et al.: Differential expression of histamine receptors in the bladder wall tissues of patients with bladder pain syndrome/interstitial cystitis – significance in the responsiveness to antihistamine treatment and disease symptoms. BMC Urol 2019; 19: 115.
13. Yamada T, Murayama T, Mita H et al.: Alternate occurrence of allergic disease and an unusual form of interstitial cystitis. Int J Urol 1998; 5: 329–335; discussion 335–336.
14. Abilov A, Ozcan R, Polat E et al.: Rare cause of dysuria: eosinophilic cystitis. J Pediatr Urol 2013; 9: e6–e8.
15. Mansouri M: Eosinophilic cystitis and interstitial cystitis: may allergy be the reason? J Ped Nephrology 2015; 3: 49–57.
16. Morfín-Maciel BM: [Correlation between renal-uretero-bladder diseases and allergies]. Rev Alerg Mex 2002; 49: 60–65.
17. Yamada T, Murayama T, Mita H et al.: Bladder hypersensitivity of interstitial cystitis complicated by allergic diseases. Urology 2001; 57 (Suppl 1): 125.
18. Tsai JD, Chen HJ, Ku MS et al.: Association between allergic disease, sleep-disordered breathing, and childhood nocturnal enuresis: a population-based case-control study. Pediatr Nephrol 2017; 32: 2293–2301.
19. Mungan NA, Seckiner I, Yesilli C et al.: Nocturnal enuresis and allergy. Scand J Urol Nephrol 2005; 39: 237–241.
20. Martín-Orozco E, Norte-Muñoz M, Martínez-García J: Regulatory T cells in allergy and asthma. Front Pediatr 2017; 5: 117.
21. Bręborowicz A: Teoria higieniczna – 2 i przeciw. Alerg Astma Immun 2006; 11 Suppl 1: 68–73.
22. Shi YH, Shi GC, Wan HY et al.: Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with allergic asthma. Chin Med J (Engl) 2011; 124: 1951–1956.
23. Ito Y, Adachi Y, Makino T et al.: Expansion of FOXP3-positive $CD4^+CD25^+$ T cells associated with disease activity in atopic dermatitis. Ann Allergy Asthma Immunol 2009; 103: 160–165.
24. Ou LS, Goleva E, Hall C et al.: T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. J Allergy Clin Immunol 2004; 113: 756–763.
25. Kalekar LA, Rosenblum MD: Regulatory T cells in inflammatory skin disease: from mice to humans. Int Immunol 2019; 31: 457–463.
26. Roesner LM, Floess S, Witte T et al.: $Foxp3^+$ regulatory T cells are expanded in severe atopic dermatitis patients. Allergy 2015; 70: 1656–1660.
27. Robinson DS, Larché M, Durham SR: Tregs and allergic disease. J Clin Invest 2004; 114: 1389–1397.
28. Grindebacke H, Wing K, Andersson AC et al.: Defective suppression of Th2 cytokines by CD4CD25 regulatory T cells in birch allergics during birch pollen season. Clin Exp Allergy 2004; 34: 1364–1372.
29. Provoost S, Maes T, Van Durme YM et al.: Decreased FOXP3 protein expression in patients with asthma. Allergy 2009; 64: 1539–1546.
30. Shi HZ, Qin XJ: CD4CD25 regulatory T lymphocytes in allergy and asthma. Allergy 2005; 60: 986–995.
31. Kraszula Ł, Pietruczuk M: Limfocyty regulatorowe CD4+ w alergicznej astmie oskrzelowej. Diagn Lab 2010; 46: 319–324.
32. Wawrzyniak A, Lipińska-Opałka A, Kalicki B et al.: The effect of passive exposure to tobacco smoke on the immune response in children with asthma. Subst Use Misuse 2021; 56: 424–430.
33. Karo-Atar D, Bitton A, Benhar I et al.: Therapeutic targeting of the interleukin-4/interleukin-13 signaling pathway: in allergy and beyond. BioDrugs 2018; 32: 201–220.
34. Gandhi NA, Pirozzi G, Graham NMH: Commonality of the IL-4/IL-13 pathway in atopic diseases. Expert Rev Clin Immunol 2017; 13: 425–437.
35. Matsunaga K, Katoh N, Fujieda S et al.: Dupilumab: basic aspects and applications to allergic diseases. Allergol Int 2020; 69: 187–196.
36. Walker C, Bauer W, Braun RK et al.: Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. Am J Respir Crit Care Med 1994; 150: 1038–1048.
37. Steinke JW, Borish L: Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. Respir Res 2001; 2: 66–70.
38. Harb H, Chatila TA: Mechanisms of dupilumab. Clin Exp Allergy 2020; 50: 5–14.
39. Izuhara K, Kanaji S, Ohta S et al.: [Clarification of the pathogenesis and development of clinical examination for allergic diseases]. Rinsho Byori 2007; 55: 369–374.
40. Nagase H, Ueki S, Fujieda S: The roles of IL-5 and anti-IL-5 treatment in eosinophilic diseases: asthma, eosinophilic granulomatosis with polyangiitis, and eosinophilic chronic rhinosinusitis. Allergol Int 2020; 69: 178–186.
41. Pelaia C, Paoletti G, Puggioni F et al.: Interleukin-5 in the pathophysiology of severe asthma. Front Physiol 2019; 10: 1514.
42. Greenfeder S, Umland SP, Cuss FM et al.: Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease. Respir Res 2001; 2: 71–79.
43. Giuffrida MJ, Valero N, Mosquera J et al.: Increased systemic cytokine/chemokine expression in asthmatic and non-asthmatic patients with bacterial, viral or mixed lung infection. Scand J Immunol 2017; 85: 280–290.
44. Zangrilli JG, Shaver JR, Cirelli RA et al.: sVCAM-1 levels after segmental antigen challenge correlate with eosinophil influx, IL-4 and IL-5 production, and the late phase response. Am J Respir Crit Care Med 1995; 151: 1346–1353.
45. Motojima S, Akutsu I, Fukuda T et al.: Clinical significance of measuring levels of sputum and serum ECP and serum IL-5 in bronchial asthma. Allergy 1993; 48 (Suppl): 98–106; discussion 110–111.
46. Polukort SH, Rovatti J, Carlson L et al.: IL-10 enhances IgE-mediated mast cell responses and is essential for the development of experimental food allergy in IL-10-deficient mice. J Immunol 2016; 196: 4865–4876.

47. Borish L, Aarons A, Rumbyrt J et al.: Interleukin-10 regulation in normal subjects and patients with asthma. J Allergy Clin Immunol 1996; 97: 1288–1296.

48. Coomes SM, Kannan Y, Pelly VS et al.: $CD4^+$ Th2 cells are directly regulated by IL-10 during allergic airway inflammation. Mucosal Immunol 2017; 10: 150–161.

49. Wilson MS, Elnekave E, Mentink-Kane MM et al.: IL-13Rα2 and IL-10 coordinately suppress airway inflammation, airway-hyperreactivity, and fibrosis in mice. J Clin Invest 2007; 117: 2941–2951.

50. Lim S, Crawley E, Woo P et al.: Haplotype associated with low interleukin-10 production in patients with severe asthma. Lancet 1998; 352: 113.

51. Kari O, Haahtela T: Conjunctival eosinophilia in atopic and non-atopic external eye symptoms. Acta Ophthalmol (Copenh) 1992; 70: 335–340.

52. Kari O: Atopic conjunctivitis. A cytologic examination. Acta Ophthalmol (Copenh) 1988; 66: 381–386.

53. Saari KM, Kari O, Haahtela T: Nonallergic eosinophilic conjunctivitis. Expert Rev Ophthalmol 2007; 2: 331–333.

54. Mierzejewska A, Jung A, Kalicki B: Nasal cytology as a marker of atopy in children. Dis Markers 2017; 2017: 4159251.

55. Sheikh J, Kaliner MA: What is the role of nasal cytology in the diagnosis of allergic rhinitis (hay fever)? Available from: https://emedicine.medscape.com/article/134825-print [cited: 2018].

56. Gelardi M, Marseglia GL, Licari A et al.: Nasal cytology in children: recent advances. Ital J Pediatr 2012; 38: 51.

57. Muriithi AK, Nasr SH, Leung N: Utility of urine eosinophils in the diagnosis of acute interstitial nephritis. Clin J Am Soc Nephrol 2013; 8: 1857–1862.

58. Nussbaum EZ, Perazella MA: Diagnosing acute interstitial nephritis: considerations for clinicians. Clin Kidney J 2019; 12: 808–813.

59. Ratnappuli A, Bissessor M, Arumugam S et al.: Culture obtained from urethral swab of asymptomatic men who screen positive for *Neisseria gonorrhoeae* by urine nucleic acid amplification testing. Sex Transm Infect 2022; 98: 139–141.

60. Wang QY, Zheng LQ, Li RH et al.: Clinician-collected urethra swab: a good alternative sample type using cobas 4800 system for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in men. J Clin Lab Anal 2020; 34: 23331.

61. Gołąb J, Jakóbisiak M, Lasek W et al.: Immunologia. Wydawnictwo PWN, Warszawa 2017: 327–361.

62. Koller DY, Halmerbauer G, Frischer T et al.: Assessment of eosinophil granule proteins in various body fluids: is there a relation to clinical variables in childhood asthma? Clin Exp Allergy 1999; 29: 786–793.

63. Klonoff-Cohen H, Polavarapu M: Eosinophil protein X and childhood asthma: a systematic review and meta-analysis. Immun Inflamm Dis 2016; 4: 114–134.

64. Wever AM, Wever-Hess J, Hermans J: The use of serum eosinophil cationic protein (ECP) in the management of steroid therapy in chronic asthma. Clin Exp Allergy 1997; 27: 519–529.

65. Morioka J, Kurosawa M, Inamura H et al.: Development of a novel enzyme-linked immunosorbent assay for blood and urinary eosinophil-derived neurotoxin: a preliminary study in patients with bronchial asthma. Int Arch Allergy Immunol 2000; 122: 49–57.

66. Nuijsink M, Hop WCJ, Sterk PJ et al.: Urinary eosinophil protein X in childhood asthma: relation with changes in disease control and eosinophilic airway inflammation. Mediators Inflamm 2013; 2013: 532619.

67. Chen Y, Yang M, Deng J et al.: Elevated levels of activated and pathogenic eosinophils characterize moderate-severe house dust mite allergic rhinitis. J Immunol Res 2020; 2020: 8085615.

68. Bouchelouche K, Kristensen B, Nordling J et al.: Increased urinary leukotriene E4 and eosinophil protein X excretion in patients with interstitial cystitis. J Urol 2001; 166: 2121–2125.

69. Storm van's Gravesande K, Mattes J, Grüntjens T et al.: Circadian variation of urinary eosinophil protein X in asthmatic and healthy children. Clin Exp Allergy 1999; 29: 1497–1501.

DOI: 10.15557/PiMR.2023.0055