2024, Retos, 51, 102-108

Reactive response of peripheral blood neutrophils of school-age adolescents with scoliosis
Resposta reativa de neutrófilos do sangue periférico de adolescentes em idade escolar com escoliose

*Dychko Dychko, *Elena Dychko, **Oleh Nekhanevych, **Vladislav Dychko, *Yulia Klimenko, *Nataliia Shaida, *Oleksandr Shaida, ***Maria Radziejowska

*Donbas State Pedagogical University (Ukraine), **Dnipro State Medical University (Ukraine), *** Czestochowa University of Technology (Poland)

Abstract. Scoliosis adversely affects the physical and mental health of children and adolescents. The scoliosis deformity develops faster during the period of intensive growth and development, which, in the absence of treatment, can lead to numerous disorders in many body systems, including the immune system. Study of the impact of the reactive response of peripheral blood neutrophils in adolescents (15-17 years old) with and without scoliosis. The reactive response of peripheral blood neutrophils of adolescents in two groups (experimental and control) was evaluated according to the indicators of the peripheral blood neutrophils reactive response rate, the neutrophil-to-lymphocyte ratio (NLR), the neutrophil, leukocyte shift ratio; the neutrophil-to-monocyte ratio: lymphocyte-to-granulocyte ratio, leukocyte to ESR (erythrocyte sedimentation rate) ratio; non-specific reactivity index. Statistical processing of the obtained results involved well-known methods of variable statistics. The peripheral blood neutrophils reactive response rate in boys with scoliosis decreased by 25.0%, while the lymphocyte-to-granulocyte ratio increased by 6.53%, the leukocyte to ESR ratio increased by 33.33%, while the neutrophil-to-monocyte ratio increased by 1.37% compared to the control group. The reactive response of peripheral blood neutrophils in adolescent girls with scoliosis is higher according to the neutrophil reactive response rate and the neutrophil shift rate by 25.0%, according to the lymphocyte-to-granulocyte ratio —by 16.97%, and lower according to the neutrophil-to-monocyte ratio by 13.10%, according to by the neutrophil-to-lymphocyte ratio — by 16.75%, according to the leukocyte shift ratio — by 16.46%, according to the leukocyte to ESR ratio — by 4.76%. It was found that the reactive response of peripheral blood neutrophils in adolescents with scoliosis, depends on gender and individual specifics of the reactive response: boys were found to have an increased reactive response of peripheral blood neutrophils was found compared to the reactive response of neutrophil granulocytes in girls. It is appropriate to continue research in this direction with the involvement of a larger category of respondents in a wider age group.
Keywords: adolescents, scoliosis, cellular reactivity, neutrophils, granulocytes.

Resumo. A escoliose afeta negativamente a saúde física e mental de crianças e adolescentes. A deformidade da escoliose se desenvolve mais rapidamente durante o período de intenso crescimento e desenvolvimento, o que, na ausência de tratamento, pode levar a inúmeras desordens em muitos sistemas do corpo, incluindo o sistema imunológico. Estudo do impacto da resposta reativa dos neutrófilos do sangue periférico em adolescentes (15-17 anos) com e sem escoliose. A resposta reativa dos neutrófilos do sangue periférico de adolescentes em dois grupos (experimental e controle) foi avaliada de acordo com os indicadores da taxa de resposta reativa dos neutrófilos do sangue periférico, a relação neutrófilo-linfócito (NLR), a razão de deslocamento de neutrófilos, leucócitos; a proporção de neutrófilos para monócitos: proporção de linfócitos para granulócitos, proporção de leucócitos para VHS (velocidade de sedimentação de eritrócitos); índice de reatividade não específico. O processamento estatístico dos resultados obtidos envolveu métodos bem conhecidos de estatística variável. A taxa de resposta reativa dos neutrófilos do sangue periférico em meninos com escoliose diminuiu em 25,0%, enquanto a proporção de linfócitos para granulócitos aumentou em 6,53%, a proporção de leucócitos para VHS aumentou em 33,33%, enquanto a proporção de neutrófilos para monócitos aumentou em 1,37 % em relação ao grupo controle. A resposta reativa dos neutrófilos do sangue periférico em meninas adolescentes com escoliose é maior de acordo com a taxa de resposta reativa de neutrófilos e a taxa de deslocamento de neutrófilos em 25,0%, de acordo com a relação linfócito-granulócito - em 16,97%, e menor de acordo com a taxa de neutrófilos - para-monócito em 13,10%, de acordo com a proporção de neutrófilos-linfócitos - em 16,75%, de acordo com a taxa de deslocamento de leucócitos - em 16,46%, de acordo com a proporção de leucócitos para ESR - em 4,76%. Verificou-se que a resposta reativa dos neutrófilos do sangue periférico em adolescentes com escoliose depende do sexo e das especificidades individuais da resposta reativa: os meninos apresentaram uma resposta reativa aumentada dos neutrófilos do sangue periférico em comparação com a resposta reativa dos granulócitos neutrófilos em meninas. É apropriado continuar a pesquisa nessa direção com o envolvimento de uma categoria maior de entrevistados em uma faixa etária mais ampla.
Palavras-chave: adolescentes, escoliose, reatividade celular, neutrófilos, granulócitos.

Resumen. La escoliosis afecta negativamente a la salud física y mental de niños y adolescentes. La deformidad escoliótica se desarrolla más rápidamente durante el período de crecimiento y desarrollo intensivo, lo que, en ausencia de tratamiento, puede provocar numerosos trastornos en muchos sistemas del cuerpo, incluido el sistema inmunológico. Estudio del impacto de la respuesta reactiva de los neutrófilos de sangre periférica en adolescentes (15-17 años) con y sin escoliosis. La respuesta reactiva de los neutrófilos de sangre periférica de adolescentes en dos grupos (experimental y de control) se evaluó de acuerdo con los indicadores de la tasa de respuesta reactiva de los neutrófilos de sangre periférica, la proporción de neutrófilos a linfocitos (NLR), la proporción de desplazamiento de neutrófilos y leucocitos; la proporción de neutrófilos a monocitos: proporción de linfocitos a granulocitos, proporción de leucocitos a VSG (velocidad de sedimentación globular); índice de reactividad no específico. El procesamiento estadístico de los resultados obtenidos involucró métodos bien conocidos de estadística variable. La tasa de respuesta reactiva de neutrófilos de sangre periférica en niños con escoliosis disminuyó en un 25,0%, mientras que la proporción de linfocitos a granulocitos aumentó en un 6,53%, la proporción de leucocitos a VSG aumentó en un 33,33%, mientras que la proporción de neutrófilos a monocitos aumentó en 1,37 % respecto al grupo control. La respuesta reactiva de los neutrófilos de sangre periférica en las adolescentes con escoliosis es mayor según la tasa de respuesta reactiva de los neutrófilos y la tasa de desplazamiento de neutrófilos en un 25,0%, según la relación linfocitos/granulocitos (en un 16,97%), y menor según la tasa de neutrófilos. - proporción de leucocitos a monocitos en un 13,10%, según la proporción de neutrófilos a linfocitos - en un 16,75%, según la proporción de desplazamiento de leucocitos - en un 16,46%, según la proporción de leucocitos a VSG - en un 4,76%. Se encontró que la respuesta reactiva de los neutrófilos de sangre periférica en adolescentes con escoliosis depende del género y de las características específicas individuales de la respuesta reactiva: se encontró que los niños tenían una mayor respuesta reactiva de los neutrófilos de sangre periférica en comparación con la respuesta reactiva de los granulocitos de neutrófilos. en niñas. Es apropiado continuar la investigación en esta dirección con la participación de una categoría más amplia de encuestados en un grupo de edad más amplio.
Palabras clave: adolescentes, escoliosis, reactividad celular, neutrófilos, granulocitos.

Fecha recepción: 04-05-23. Fecha de aceptación: 01-09-23
Vladislav Dychko
vladyslavchernii2@gmail.com

Introduction

Neutrophils are short-lived cells making up to 95% of the total number of peripheral blood leukocytes. Neutrophils are extremely sensitive to the slightest changes in homeostasis, are able to quickly leave the bloodstream to capture and destroy invading microorganisms (Dychko, 2018; Dychko et al., 2018; Hayashi et al., 2003). The huge microbiocidal and cytolytic potential enables them to effectively neutralize and eliminate pathogenic and conditionally pathogenic microorganisms (Semianiv et al., 2022; Sydorchuk et al., 2022).

In current experimental studies neutrophils are considered not only as effector cells. They are capable of exerting a significant regulatory influence on other blood cells, epithelial cells and connective tissue, as well as on plasma enzyme systems. Upon activation, neutrophils, together with granule products, produce a wide range of cytokines, thereby not only influencing the activity of other immune cells (ICs), but also regulate the immune response. Different events associated with the expression of genes that encode multiple transcription factors, as well as regulate protein synthesis and stable cytokine production are observed in activated normally differentiated short-lived neutrophils (Kabayashi et al., 2004; Sydorchuk et al., 2015).

Despite the significant progress in studying the neutrophil reactivity and the mechanisms underlying their reactive response, immunology and biology still lack studies on the reactivity of neutrophils and anti-inflammatory compounds. One of the areas of research on this issue is the study of the mutual influence of pathological spine disorders on the general condition of the immune system and the neutrophil reactivity in different age groups (Cameron-Christie et al., 2018; Campbell et al., 2018; Gernez et al., 2018, Li et al., 2019; Nebert & Liu, 2019; Pearson et al., 2019; Sandler & Hayes-Jordan, 2018; Teixeira et al., 2021a). For example, Teixeira et al. (2021b) showed the relationship between the condition of the immune system in adolescents with intervertebral disc disease (Teixeira, 2021), while Mitchell et al. (2021) studied the possibility of premature cervical spine degeneration in people with Job's syndrome.

This direction of research reveals that the problem is not sufficiently covered and requires a broad in-depth study. Special attention should be paid to studies of the adolescents (13-19 years), as the primary age of scoliosis is 10-15 years, which occurs equally in people of both genders.

The aim of the research is to study the impact of the reactive response of peripheral blood neutrophils in adolescents (15-17 years old) with and without scoliosis.

Materials and methods

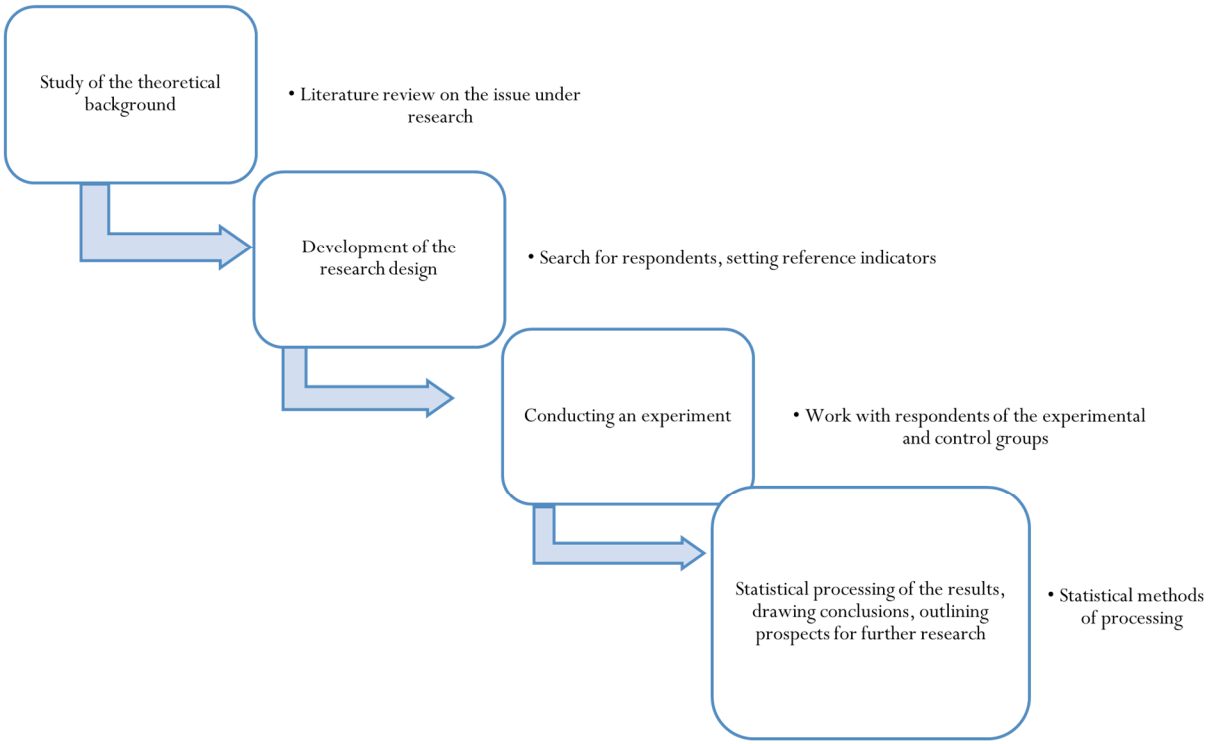

Figure 1. General methodology for the study of the reactive response of peripheral blood neutrophils of school-age adolescents with scoliosis

Selection of the respondents

The respondents of the study were adolescents with scoliosis and generally healthy boys and girls of 15-17 years. The total sample was divided into 2 groups of respondents: experimental and control.

The experimental group included 24 adolescents (13 boys and 11 girls) of the Oleksiievo-Druzhkivska General Educational Sanatorium Boarding Level I-III School No. 13 for children with scoliosis.

The control group included 30 adolescents (15 boys and

girls each) from the Oleksiievo-Druzhkivska Secondary School No. 17.

Setting reference indicators

The reactive response of neutrophils in the peripheral blood of adolescents was evaluated according to the following indicators: the peripheral blood neutrophils reactive response rate, the neutrophil-to-lymphocyte ratio, the neutrophil, leukocyte shift ratio; the neutrophil-to-monocyte ratio: lymphocyte-to-granulocyte ratio, leukocyte to ESR ratio; non-specific reactivity index.

Statistical processing of the results

The statistical processing of the obtained results was carried out using the well-known methods of variable statistics with the calculation of the means (M) and standard error of the mean (±m), the reliability of data for independent samples was calculated by using Student's t-test. The difference was considered significant at $p<0.05$.

Ethical criteria

The work was carried out in accordance with the provisions of the Council of Europe Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (1997), the Ethical Principles for Medical Research Involving Human Subjects adopted by the 52nd Assembly of the World Medical Association (2000), Universal Declaration on Bioethics and Human Rights adopted by the resolution of the UNESCO General Conference (2005), the Declaration of Helsinki principles (1964), and in compliance with the current regulatory requirements of Ukraine.

At the beginning of the study, the parents of all children gave their informed written consent to the examination of their children and the use of the obtained data in the research.

Results

Table 1 presents the results of the study of the indicators of the reactive response of the peripheral blood neutrophils in adolescents of the experimental and control groups.

Table 1.
Reactive response of peripheral blood neutrophils in adolescents

Indicators	Measurement units	Control group			Experimental group					P1	P2
		Gender			Gender						
		Boys (n = 15)	Girls (n = 15)	P	Boys (n = 13)		Girls (n = 11)				
					indicators	DID	indicators	DID	P		
Neutrophils Reactive response rate	c.u.	0.05±0.002	0.03±0.002	<0.05	0.04±0.001	-I	0.05±0.002	+II	<0.05	<0.05	<0.05
Neutrophil shift ratio	c.u.	0.05±0.002	0.03±0.001	<0.05	0.04±0.002	-I	0.05±0.001	+II	<0.05	<0.05	<0.05
Neutrophil-to-lymphocyte ratio	c.u.	10.23±1.02	11.05±1.07	>0.05	10.37±1.02	+I	9.77±0.93	-I	>0.05	>0.05	>0.05
Neutrophil to lymphocyte ratio	c.u.	2.43±0.12	2.23±0.10	>0.05	2.31±0.09	-I	1.91±0.07	-I	<0.05	>0.05	<0.05
Leukocyte shift ratio	c.u.	2.03±0.03	1.91±0.02	<0.05	1.93±0.02	-I	1.64±0.02	-I	<0.01	<0.05	<0.01
Lymphocyte-to-granulocyte ratio	c.u.	3.98±0.07	4.36±0.09	<0.05	4.24±0.08	+I	5.10±0.09	+I	<0.01	<0.05	<0.01
Leukocyte to ESR (erythrocyte sedimentation rate) ratio	c.u.	0.75±0.05	0.88±0.06	>0.05	1.00±0.97	+I	0.84±0.05	-I	>0.05	<0.05	>0.05
Non-specific reactivity index	c.u.	2.31±0.04	2.16±0.03	<0.05	2.23±0.03	-I	0.82±0.02	-I	<0.01	>0.05	<0.01

Note: DID - degree of immune disorders. P - the reliability of the differences in the indicators of boys and girls within the group; P1 - reliability of differences between the indicators of boys of both groups; - P2 - reliability of differences between indicators of the control and experimental groups.

It is settled that in the control group of conditionally healthy adolescents the value of neutrophils reactive response rate as well as neutrophil shift ratio and leucocyte shift ratio are statistically higher in boys compared to girls (table 1). Data of neutrophil-to-lymphocyte ratio, lymphocyte-to-granulocyte ratio, leucocyte-to-ESR ratio and non-specific reactivity index merely have no significant differences depending on gender (Table 1).

While comparing results of control group and group of adolescents with scoliosis, one can reveal different character of changes. Thus, in boys neutrophils reactive response rate decreases up to 0.04±0.001 c.u. in comparison with conditionally healthy boys (0.05±0.002 c.u.) and it appears to be lower than in the group of girls with scoliosis (0.05±0.002 c.u.). Neutrophil shift ratio changes in the same manner (Table 1).

While looking at the dynamic of neutrophil-to-lymphocyte ratio in all groups of investigated adolescents, one can see that in male group it doesn't change, however in female group it shows tendency to decreasing (Table 1). Leukocyte shift ratio is hardly ever changed in comparison with data of conditionally healthy adolescents. One can see only a slight

tendency for decreasing (Table 1). Vice versa, lymphocyte-to-granulocyte ratio imperceptibly increases both in male and female groups, compared to control groups of adolescents. Leukocyte- to- ESR ratio fractionally changes: in boys it slightly increase and in girls it insignificantly decreases (Table 1). Non-specific reactivity index authentically decreases only in female group of adolescents with scoliosis. In male group it doesn't change in comparison with control group.

In general, it is possible to say that the most reliable indexes of neutrophils reactivity in adolescents with scoliosis are neutrophils reactive response rate, neutrophil shift ratio, appropriate both to male and female groups and non-specific reactivity index inherent to female group of adolescents with scoliosis (Table 1). When comparing the indicators of the experimental and control groups with distribution by gender, the differences in indicators are heterogeneous: in the experimental group of boys, the indicators of the lymphocyte-to-granulocyte ratio and the index of leukocyte to ESR (erythrocyte sedimentation rate) ratio prevail, while in girls — the neutrophils reactive response rate, neutrophil shift index and lymphocyte-to-granulocyte ratio. It attracts attention that neutrophils reactive response rate and neutrophil shift ratio demonstrate the opposite trend of dynamics. This, as well as changes in non-specific reactivity index, may be explained by different action of sex hormones and their influence on activity of white blood cells in the case of scoliosis.

Discussion

Adolescent scoliosis takes the first place among idiopathic scoliosis in children and forms approximately 90 % of cases. It usually appears at the age of 11–18 years (Konieczny et al., 2013). The prevalence of scoliosis in girls was reported by Daruwalla et al. (1985), the ratio girls to boys was of 2:1, with age it rises up to 3:1. Cilli et al. (2009) and Nery et al. (2010) didn't find difference in age groups however they revealed a prevalence ratio female to male of 2:1. More than that, some authors (Suh et al., 2011; Daruwalla et al., 1985) pay attention to the issue of scoliosis severity, mentioning that in girls it progresses to a higher grade of severity. Meanwhile in the investigation of Wang et al. (2012) one can see that atypical curve types prevails in boys compared to girls. All these differences in severity and prevalence of scoliosis depending on sex and age are mainly conditioned by hormone repertoire, expressing of receptors to sex hormones on bone cells structures and on other variety of factors, because we cannot look at bones or skeletal system in general as on the isolated system. Both in women and men estrogen turns to be one of the major hormonal regulator of bone metabolism by its connection with estrogen receptors which are highly expressed on bone cells membranes. Due to this estrogen provides a pronounced influence on bone turnover and fulfils this also through interaction with immune system (Khosla et al., 2012).

Estrogens and androgens as other steroid hormones influence on activity of immune system and modulate the immune response depending on gender peculiarities (Klein & Flanagan, 2016). It was established that in adolescents the value of indicators of the peripheral blood neutrophils reactive response is gender-dependent. This fact is consistent with the data Ratajczak-Wrona et al. (2021) showing gender dependence of neutrophil response by bisphenol A.

It is settled that in the boys of the control group, the reactive response rate and neutrophil shift ratio is higher than that of girls — by 66.67%, the leukocyte shift ratio — by 6.29%, and the non-specific reactivity index — by 6.94%. At the same time, in girls of the control group, the lymphocyte-to-granulocyte ratio is higher by 9.55%, and there is a tendency towards an increasing neutrophil-to-monocyte ratio by 8.02%. This fact may be explained by the influence of sex hormones on activity of immune cells and as consequence on inflammation in general. Male and female hormones provide different effect on immune cells including neutrophils depending on estrogen receptors expression level on their membranes (Bereshchenko et al., 2018).

The peripheral blood neutrophils reactive response rate in the boys of the experimental group compared to the indicators of respondents of the control group decreased by 25.0%, the neutrophil-to-lymphocyte ratio - by 5.19%, the leukocyte shift ratio - by 5.18%, and non-specific reactivity index - by 3.59%. At the same time, in the boys of the experimental group, the lymphocyte-to-granulocyte ratio increased by 6.53%, the leukocyte to ESR (erythrocyte sedimentation rate) ratio - by 33.33%, and the neutrophil-to-monocyte ratio - by 1.37%, compared to the same indicators of the respondents of the control group. Obtained data show that scoliosis includes not only musculoskeletal definitions but also some impairments from the side of immune system, it is not a paradox. Papanastasiou et al. (2010) shown that hyper-IgE syndrome, manifests by frequent inflammation because of bacterial infection, was typical for idiopathic scoliosis. More than that, Kartalija et al. (2013) settled that idiopathic-like scoliosis in 50% of patients was followed by recurring nontuberculosis mycobacterial.

Changes in white blood cells contents and indexes were also revealed in girls with scoliosis. So, the peripheral blood neutrophils reactive response of girls in the experimental group compared to the control group is higher according to the neutrophils reactive response rate and the neutrophil shift ratio by 25.0%, according to the lymphocyte-to-granulocyte ratio — by 16.97% and lower, according to the index of the neutrophil-to-monocyte ratio — by 13.10%, according to the neutrophil-to-lymphocyte ratio — by 16.75%, according to the leukocyte shift ratio — by 16.46%, according to the leukocyte to ESR (erythrocyte sedimentation rate) ratio — by 4.76%. These changes in ratios indicate a decrease in the non-specific anti-infective protection index by 18.68%.

Compared to girls, the boys of the experimental group had decreased neutrophils reactive response rate and neutrophil shift index by 25.0%, and the lymphocyte-to-

granulocyte ratio — by 20.28%. However, in boys of the experimental group, compared to girls, the neutrophil-to-monocyte ratio increased by 6.14%, the neutrophil-to-lymphocyte ratio — by 20.34%, the leukocyte shift ratio — by 17.68%, the leukocyte to ESR (erythrocyte sedimentation rate) ratio — by 19.05%. These changes in the boys of the experimental group contributed to an increase in the non-specific anti-infective protection index (non-specific reactivity of the body) by 22.53% compared to girls.

Revealed data concerning peculiarities of reactive response of peripheral blood neutrophils in adolescents with scoliosis underline interrelations between immune and bony systems and stress the presence of inflammation in patients with scoliosis, which in its turn may be primary or secondary. Osteoimmunology is one of immunology branches which help scientists to understand a holistic nature of the body and the presence of interconnections between all processes, impairments and diseases, which take place in the exact organism (Guder et al., 2020; Ponzetti & Rucci, 2019). In general, the development of osteoporosis which is usually revealed in patients with idiopathic scoliosis, is tightly stipulated by the immune system impairment and inflammatory (Limmer & Wirtz, 2017; Guder et al., 2020). More than that there is a report by Huang, et al. (2018), which pay attention to the monocyte- to-lymphocyte ratio, as a marker of systemic inflammation, which, in its turn, according to Ulucaköy et al. (2020) and Gao et al. (2019), has been associated with bone diseases including osteoporosis. More than that, it was also proved that neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio, known as systemic inflammatory response markers (Gary et al., 2013), are associated with low Bone mineral density (Koseoglu, 2017; Öztürk et al., 2013).

Revealed difference in increased neutrophils reactive response in girls compared to boys is more likely caused by inhibition effect of androgens on cells of innate immunity (Roberts et al. 2001) which takes place in male puberty due to increasing of androgens level. In general, to reveal measures for prevention of scoliosis progression in boys and girls, its connection with immune response it is necessary, first, to define its aetiology, whether it is congenital, idiopathic or neuromuscular one. On one hand physical activity is essential for scoliosis prevention, but on the other there were not verified data concern this postulate (Barreto et al., 2023; Tobias et al., 2019), etc. That is why, despite familiarity of scoliosis, this problem needs comprehensive investigation including influence of prenatal factors, food habits, intensity of physical trainings, etc., which may clarify the situation and help to elaborate preventive measures.

Research limitations

The sample of respondents consists of a relatively small number of participants because of the initial stage of research. The aetiology of scoliosis was not taken in consideration. Sex hormones and cortisol blood concentration was not considered.

Conclusions

The peripheral blood neutrophils reactive response rate in adolescents is age-dependent. With age, it increases or tends to increase in adolescent girls and boys with scoliosis. The neutrophils reactive response is also gender-dependent — in adolescent girls with scoliosis compared to this indicator in adolescent boys with scoliosis, the neutrophils reactive response increases due to an increase in the neutrophils reactive response rate, neutrophil shift index, lymphocyte-to-granulocyte ratio, neutrophil-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio. At the same time, the non-specific reactivity of the body of boys with scoliosis has higher indicators than that of girls with scoliosis.

Prospects for further research

The research results can be used in the assessment of the mutual influence of spine pathologies and the state of the immune system of adolescents. It is promising to conduct similar studies with a larger number of respondents and a wider range of age groups. Reactive response of peripheral blood neutrophils in adolescents with scoliosis will be examined depending on scoliosis aetiology and sex hormones and cortisol blood concentration.

References

Barreto, A. C., Medeiros, A. P., Araujo, G. da S., Vale, R., Vianna, J. M., Alkimin, R., ... & Novaes, J. da S. (2023). Heart rate variability and blood pressure during and after three CrossFit® sessions. *Retos, 47*, 311-316. https://doi.org/10.47197/retos.v47.93780

Bereshchenko, O., Bruscoli, S., & Riccardi, C. (2018). Glucocorticoids, sex hormones, and immunity. *Frontiers in Immunology*, *9*, 1332. https://doi.org/10.3389/fimmu.2018.01332

Cameron-Christie, S. R., Wells, C. F., Simon, M., Wessels, M., Tang, C. Z. N., Wei, W., ... & Krakow, D. (2018). Recessive Spondylocarpotarsal Synostosis Syndrome Due to Compound Heterozygosity for Variants in MYH3. *American Journal of Human Genetics, 102*(6), 1115-1125. https://doi.org/10.1016/j.ajhg.2018.04.008

Campbell, I. M., Sheppard, S. E., Crowley, T. B., McGinn, D. E., Bailey, A., McGinn, M. J., ... & Zackai, E. H. (2018). What is new with 22q? An update from the 22q and You Center at the Children's Hospital of Philadelphia. *American Journal of Medical Genetics Part A, 176*(10), 2058-2069. https://doi.org/10.1002/ajmg.a.40637

Cilli, K., Tezeren, G., Taş, T., Bulut, O., Oztürk, H., Oztemur, Z., & Unsaldi, T. (2009). School screening for scoliosis in Sivas, Turkey. *Acta Orthopaedica et Traumatologica Turcica*, *43*(5), 426-430. https://doi.org/10.3944/AOTT.2009.426

Daruwalla, J. S., Balasubramaniam, P., Chay, S. O., Rajan,

U., & Lee, H. P. (1985). Idiopathic scoliosis. Prevalence and ethnic distribution in Singapore schoolchildren. *The Journal of Bone & Joint Surgery British Volume*, *67*(2), 182-184. https://doi.org/10.1302/0301-620X.67B2.3980521

Dychko, O. (2018). Influencing scoliosis on reactive neurophilic granulocytes in peripheral blood in children aged 11-14. *Bulletin of Problems of Biology and Medicine: Ukrainian Scientific and Practical Journal, 2*(4(147)), 377-379. Available from: https://vpbm.com.ua/en/vyipusk-4-tom-2-(147),-2018/11724

Dychko, V. V., Sheyko, V. I., Bobirev, V. E., & Vasylevskyi, V. S. (2018). Cellular reactivity of the body of children aged 7-10 years with visual pathology. *Bulletin of Problems of Biology and Medicine, 2*(4(147)), 380-382. http://dx.doi.org/10.29254/2077-4214-2018-4-2-147-380-382

Gao, K., Zhu, W., Liu, W., Ma, D., Li, H., Yu, W., Li, Q., & Cao, Y. (2019). The predictive role of monocyte-to-lymphocyte ratio in osteoporosis patient. *Medicine*, *98*(34), e16793. https://doi.org/10.1097/MD.0000000000016793

Gary, T., Pichler, M., Belaj, K., Hafner, F., Gerger, A., Froehlich, H., … & Brodmann, M. (2013). Platelet-to-lymphocyte ratio: A novel marker for critical limb ischemia in peripheral arterial occlusive disease patients. *PloS One*, *8*(7), e67688. https://doi.org/10.1371/journal.pone.0067688

Gernez, Y., Freeman, A. F., Holland, S. M., Garabedian, E. R. N., Patel, N. C., Puck, J. M., … & Chan, A. Y. (2018). Autosomal Dominant Hyper-IgE Syndrome in the USIDNET Registry. *The Journal of Allergy and Clinical Immunology: In Practice, 6*(3), 996-1001. https://doi:10.1016/j.jaip.2017.06.041

Guder, C., Gravius, S., Burger, C., Wirtz, D. C., & Schildberg, F. A. (2020). Osteoimmunology: A current update of the interplay between bone and the immune system. *Frontiers in Immunology, 11*. https://doi.org/10.3389/fimmu.2020.00058

Hayashi, F., Means, T. K., & Luster, A. D. (2003). Toll-like receptors stimulate human neutrophil function. *Blood, 102*(7), 2660-2669. https://doi.org/10.1182/blood-2003-04-1078

Huang, Y., Deng, W., Zheng, S., Feng, F., Huang, Z., Huang, Q., … & Li, T. (2018). Relationship between monocytes to lymphocytes ratio and axial spondyloarthritis. *International Immunopharmacology*, *57*, 43-46. https://doi.org/10.1016/j.intimp.2018.02.008

Kabayashi, S. D., Vogish, I. M., Branshton, K. R., Whitney, A. R., Nauseef, W. M., Malech, H. L., & DeLeo, F. R. (2004). Gene expression profiling provides insight into the pathophysiology of chronic granulomatous disease. *Journal of Immunology, 172*(1), 636-643. https://doi.org/10.4049/jimmunol.172.1.636

Kartalija, M., Ovrutsky, A. R., Bryan, C. L., Pott, G. B., Fantuzzi, G., Thomas, J., … & Chan, E. D. (2013). Patients with nontuberculous mycobacterial lung disease exhibit unique body and immune phenotypes. *American Journal of Respiratory and Critical Care Medicine*, *187*(2), 197-205. https://doi.org/10.1164/rccm.201206-1035OC

Khosla, S., Oursler, M. J., & Monroe, D. G. (2012). Estrogen and the skeleton. *Trends in Endocrinology and Metabolism: TEM*, *23*(11), 576-581. https://doi.org/10.1016/j.tem.2012.03.008

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews. Immunology*, *16*(10), 626–638. https://doi.org/10.1038/nri.2016.90

Konieczny, M. R., Senyurt, H., & Krauspe, R. (2013). Epidemiology of adolescent idiopathic scoliosis. *Journal of Children's Orthopaedics*, *7*(1), 3-9. https://doi.org/10.1007/s11832-012-0457-4

Koseoglu, S. B. (2017). Bone loss & platelet-to-lymphocyte ratio. *Biomarkers in Medicine*, *11*(1), 5-10. https://doi.org/10.2217/bmm-2016-0188

Li, J., Li, N., Chen, Y., Hui, S., Fan, J., Ye, B., Fan, Z., et al. (2019). SPRY4 is responsible for pathogenesis of adolescent idiopathic scoliosis by contributing to osteogenic differentiation and melatonin response of bone marrow-derived mesenchymal stem cells. *Cell Death & Disease, 10*(11), 805. https://doi:10.1038/s41419-019-1949-7

Limmer, A., & Wirtz, D. C. (2017). Osteoimmunology: Influence of the immune system on bone regeneration and consumption. *Zeitschrift fur Orthopadie und Unfallchirurgie*, *155*(3), 273-280. https://doi.org/10.1055/s-0043-100100

Mitchell, A. L., Urban, A. K., Freeman, A. F., & Hammoud, D. A. (2021). An unusual pattern of premature cervical spine degeneration in STAT3-LOF. *Journal of Clinical Immunology, 41*, 576-584. https://link.springer.com/article/10.1007/s10875-020-00926-z

Nebert, D. W., & Liu, Z. (2019). SLC39A8 gene encoding a metal ion transporter: discovery and bench to bedside. *Human Genomics, 13*(S1), 51. https://humgenomics.biomedcentral.com/articles/10.1186/s40246-019-0233-3

Nery, L. S., Halpern, R., Nery, P. C., Nehme, K. P., & Stein, A. T. (2010). Prevalence of scoliosis among school students in a town in southern Brazil. *Sao Paulo Medical Journal = Revista Paulista de Medicina*, *128*(2), 69-73. https://doi.org/10.1590/s1516-31802010000200005

Öztürk, Z. A., Yesil, Y., Kuyumcu, M. E., Bilici, M., Öztürk, N., Yeşil, N. K., … & Arıoğul, S. (2013). Inverse relationship between neutrophil lymphocyte ratio (NLR) and bone mineral density (BMD) in elderly people. *Archives of Gerontology and Geriatrics*, *57*(1), 81-85. https://doi.org/10.1016/j.archger.2013.02.005

Papanastasiou, A. D., Mantagos, S., Papanastasiou, D. A., & Zarkadis, I. K. (2010). A novel mutation in the signal transducer and activator of transcription 3 (STAT3) gene, in hyper-IgE syndrome. *Molecular Immunology*, *47*(7-8),

1629-1634. https://doi.org/10.1016/j.molimm.2010.01.010

Pearson, M. J., Philp, A. M., Haq, H., Cooke, M. E., Nicholson, T., Grover, L. M., ... & Hammond, C. L. (2019). Evidence of intrinsic impairment of osteoblast phenotype at the curve apex in girls with adolescent idiopathic scoliosis. *Spine Deformity, 7*(4), 533-542. https://doi.org/10.1016/j.jspd.2018.11.016

Ponzetti, M., & Rucci, N. (2019). Updates on osteoimmunology: What's new on the cross-talk between bone and immune system. *Frontiers in Endocrinology, 10*. https://doi.org/10.3389/fendo.2019.00236

Ratajczak-Wrona, W., Garley, M., Rusak, M., Nowak, K., Czerniecki, J., Wolosewicz, K., ... & Jablonska, E. (2021). Sex-dependent dysregulation of human neutrophil responses by bisphenol A. *Environmental Health: A Global Access Science Source*, *20*(1), 5. https://doi.org/10.1186/s12940-020-00686-8

Roberts, C. W., Walker, W., & Alexander, J. (2001). Sex-associated hormones and immunity to protozoan parasites. *Clinical Microbiology Reviews*, *14*(3), 476-488. https://doi.org/10.1128/CMR.14.3.476-488.2001

Sandler, G., & Hayes-Jordan, A. (2018). Chest wall reconstruction after tumor resection. *Seminars in Pediatric Surgery, 27*(3), 200-206. https://doi.org/10.1053/j.sempedsurg.2018.05.008

Semianiv, M., Sydorchuk, L., Semianiv, I., & Sydorchuk, R. (2022). Dyslipidemia as a predictor of essential arterial hypertension depending on AGTR1 (RS5186) and VDR (RS2228570) genes polymorphism. *Romanian Journal of Diabetes Nutrition and Metabolic Diseases, 29*(2), 253-260. https://rjdnmd.org/index.php/RJDNMD/article/view/1156

Suh, S. W., Modi, H. N., Yang, J. H., & Hong, J. Y. (2011). Idiopathic scoliosis in Korean schoolchildren: A prospective screening study of over 1 million children. *European Spine Journal*, *20*(7), 1087-1094. https://doi.org/10.1007/s00586-011-1695-8

Sydorchuk, A. R., Sydorchuk, L. P., Gutnitska, A. F., Dzhuryak, V. S., Kryvetska, I. I., Sydorchuk, R. I., Ursuliak, Y. V., & Iftoda, O. M. (2022). Endothelium function regulation markers and carotid intima-media thickness in arterial hypertension depending on NOS3 (rs2070744) and GNB3 (rs5443) genes polymorphism: a prospective case-control study. *Endocrine Regulations, 56*(2), 104-114. https://doi.org/10.2478/enr-2022-0012

Sydorchuk, I. Y., Sydorchuk, L. I., Levytska, S. A., Kaspruk, N. A., Sydorchuk, R. I., Sydorchuk, L. P., & Sydorchuk, A. S. (2015). Reactive response of neutrophils of peripheral blood of patients with acute bronchitis. *Bukovinian Medical Herald, 19*(2(74)), 172-178. https://doi.org/10.24061/2413-0737.XIX.2.74.2015.105

Teixeira, G. Q., Yong, Z., Goncalves, R. M., Kuhn, A., Riegger, J., Brisby, H., Henriksson, H. B., & Le Maitre, C. L. (2021a). Terminal complement complex formation is associated with intervertebral disc degeneration. *European Spine Journal, 30*(1), 217-226. https://doi.org/10.1007/s00586-020-06592-4

Teixeira, G. Q., Yong, Z., Kuhn, A., Riegger, J., Goncalves, R. M., Ruf, M., ... & Le Maitre, C. L. (2021b). Interleukin-1β and cathepsin D modulate formation of the terminal complement complex in cultured human disc tissue. *European Spine Journal, 30*(8), 2247-2256. https://doi.org/10.1007/s00586-021-06901-5

Tobias, J. H., Fairbank, J., Harding, I., Taylor, H. J., & Clark, E. M. (2019). Association between physical activity and scoliosis: A prospective cohort study. *International Journal of Epidemiology*, *48*(4), 1152-1160. https://doi.org/10.1093/ije/dyy268

Wang, W., Zhu, Z., Zhu, F., Sun, C., Wang, Z., Sun, X., & Qiu, Y. (2012). Different curve pattern and other radiographical characteristics in male and female patients with adolescent idiopathic scoliosis. *Spine*, *37*(18), 1586-1592.https://doi.org/10.1097/BRS.0b013e3182511d0c