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ANATOMICAL AND ULTRASOUND STUDY OF THE MID-FEMORAL SCIATIC NERVE AND ITS DIVISION

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Abstract

OBJECTIVES: Anatomical variations of the sciatic nerve were supposed as potential causes for incomplete blocks at the level of the popliteal fossa. Therefore, we aimed to conduct an anatomical and ultrasound survey of the mid-femoral sciatic nerve and its division.
BACKGROUND: A regional block of the sciatic nerve is a procedure for analgesia and anaesthesia of the lower extremity. Various approaches to the sciatic nerve are used in clinical practice. However, the sciatic nerve demonstrates several variations regarding its topography and division.
MATERIALS AND METHODS: The anatomical study included twenty lower limbs of ten adult cadavers. The ultrasound study involved ten upper legs of five healthy volunteers
RESULTS: The sciatic nerve was found distally to the piriformis muscle in all our cadaveric specimens. It was divided into two major branches (common peroneal nerve and tibial nerve) at a mean distance of 68.1 ± 19.3 mm above the popliteal crease. The observed distances ranged widely from 35 to 113 mm. The mid-femoral sciatic nerve and its division were entirely revealed using ultrasound in all volunteers.
CONCLUSION: The sciatic nerve presents significant anatomical variations, which may cause an incomplete block. Understanding ultrasound anatomy and ultrasound appearance of the sciatic nerve is essential for performing ultrasound-guided sciatic blocks.

Keywords: anatomy; dissection; nerve block; sciatic nerve; ultrasonography

INTRODUCTION

Regional blockade of the sciatic nerve (SN) is a commonly used method for providing analgesia and anaesthesia to the lower extremity and there are several different approaches to SN. The rate of success for all procedures is generally 90–95%, with about 5 % of cases necessitating additional general anaesthesia. An incomplete block is thought to be the outcome of defective diffusion (due to SN size), unconnected epineural fascial coverings of the common peroneal nerve (CPN) and tibial nerve (TN), or a block of a single SN component. SN block is frequently performed in the popliteal fossa (PF) at the area of the bifurcation of the SN. The applications of local anaesthetics close to the division of SN have the advantage of being relatively superficial and allowing the anaesthetics to be distributed to CPN and TN. The name of this procedure is popliteal block. A popliteal block anaesthetizes the whole leg distally to the proximal epiphysis of the tibia except for the medial surface of the foot and calf. The needle tip must be ideally positioned close to the SN main trunk. If the local anaesthetic is applied too distal, the probability of an incomplete block increases because the two main branches of the SN have separated (1–4).

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Anatomy

SN is a mixed nerve, the largest in the human body. Motor fibres innervate the dorsal group of the muscles of the thigh, including semimembranosus, semitendinosus, and biceps femoris. They also supply the ischial part of the adductor magnus and all muscles below the knee (5). Sensory fibres of the SN innervate the skin of the foot and lower leg, except for the supply of the medial region of the calf and the medial margin of the foot, which is provided by the saphenous nerve (6). SN comes from the sacral plexus and consists of ventral branches of the fourth lumbar to a third sacral spinal nerve. The ventral branches of these spinal nerves are positioned on the piriformis muscle's anterior surface. SN exited the pelvis via the greater sciatic foramen, generally under the piriformis. It then continues along the thigh's posterior surface medially to the greater trochanter and laterally to the ischial tuberosity (7). Two nerves of the sacral plexus, CPN and TN, form together SN. They are bound together initially by connective tissue; TN lies anteriorly and medially, and the CPN is located more laterally and posteriorly. Within the SN, both components are surrounded by their own epineural sheaths and separated from each other by a thin Compton – Cruveilhier septum. The SN is situated on the posterior surface of the adductor magnus, deep to the biceps femoris, when it penetrates the thigh and descends to the PF. In the upper edge of the PF, the SN is bordered medially by the tendons of semimembranosus and semitendinosus and laterally by the tendon of the long head of the biceps femoris. SN is located lateral and posterior to the popliteal artery and popliteal vein in the upper part of the PF. It usually splits itself into its component nerves, CPN and TN, within the apex of the PF (1,8,9). The TN runs parallel to the popliteal vessels and straight caudally, deep inside the PF. Following its separation superficially and laterally, the CPN continues parallel to the biceps femoris distal tendon (10).

MATERIALS AND METHODS

Both studies were realized at the Faculty of Medicine of Pavol Jozef Šafárik University in Košice. An anatomical survey was conducted on twenty lower limbs of ten adult cadavers during undergraduate dissections for first-year general medicine students. All cadavers were under the administration of the Department of Anatomy and were acquired from the body donation program after signing the informed consent of the donors themselves. Of these, five were males and five were females. All cadavers were without the finding of obvious pathology and were preserved with a formalin-based solution soon after death. For the purpose of our research, the popliteal crease (PC) was found, the skin with the subcutaneous tissue at the level of PF was taken off, and further dissection was performed to identify CPN and TN. Next, a posterior thigh dissection was performed to discover the position of the SN, and a location of bifurcation was noticed. The division of the SN was defined as the point where the epineural sheath of the SN was divided into the separate epineural sheaths of its terminal branches. Finally, a dissection of the gluteal region was also fulfilled and the nerve's exit from the pelvis and its relation to piriformis was recorded. For each dissected lower extremity, the distance between the bifurcation of the SN and PC was measured. Metric analyses were performed using a sliding calliper and the possible error was estimated at 0.5 mm. A paired t-test was run to compare the distances from the SN division to the PC between the sides of the lower limbs (right vs left) and between sexes (male vs female). Statistical significance was determined by p-values less than 0.05.

An ultrasound survey of the SN location was carried out using an ultrasound unit LOGIQ V2, GE Healthcare Systems, United States, at the First Department of Anaesthesiology and Intensive Medicine. After written informed consent, five healthy adult volunteers (2 males, 3 females, 33–55 years old, 55–90 kg, 162–185 cm) participated in this study. The volunteers were staff members of the same department. The exclusion criteria were upper leg skin inflammation, lower limb pain, and immobility. Ultrasound scans were performed from

the dorsal approach in a prone position using a linear transducer array with the frequencies of 8 to 13 MHz by a radiologist and an anaesthesiologist with 10 and 25 years of experience, respectively. In all volunteers, two thighs were examined and the SN was systematically scanned in the cross-section from the midthigh to the PF.

RESULTS

The results of the anatomical study are presented using mean and standard deviation.

Ten right and ten left thighs from five male and five female cadavers were examined. The SN, CPN, and TN were identified in all dissected lower extremities (Fig. 1). The SN exited the pelvis below the piriformis in all our specimens (type A in the Beaton and Anson classification system). It was divided at a mean distance of 68.1 ± 19.3 mm above the PC. The measured distances ranged from 35 to 113 mm (Fig. 2). There was no statistical difference in the measured distances by sides in all cadaver legs (69.8 ± 17.9 mm vs 66.4 ± 22.4 mm for right and left sides, respectively; p=0.712). In addition, the measured distances did not differ between the sexes (69.4 ± 24.7 mm vs 70.2 ± 10.5 mm for right male and female lower limbs, respectively; p=0.948 and 64.4 ± 17.6 mm vs 68.4 ± 28.3 mm for left male and female lower limbs, respectively, p=0.795).

The SN was clearly seen by ultrasound as a round to oval hyperechoic formation with a fascicular structure. In the mid femoral region, that is in the second third of the distance from the greater trochanter to the PC, SN was located dorsally from the adductor magnus and ventrally from the long head of biceps femoris (Fig. 3). The popliteal part of the SN was found superficially and laterally to the main vessels in the PF. The most medial and most deep-seated was the popliteal artery. Over it was the popliteal vein, and most superficially was positioned the SN. It was divided here into the CPN (lateral) and TN (medial) (Fig. 4). In all volunteers, the SN division and proximal parts of the CPN and TN were entirely revealed using ultrasound. The internal appearance of the nerves in short-axis visualization was seen as honeycomb hypoechoic areas surrounded by hyperechoic structures. The hypoechoic portions represent nerve tissue, whereas the hyperechoic regions correspond to fibrous and adipose tissue.

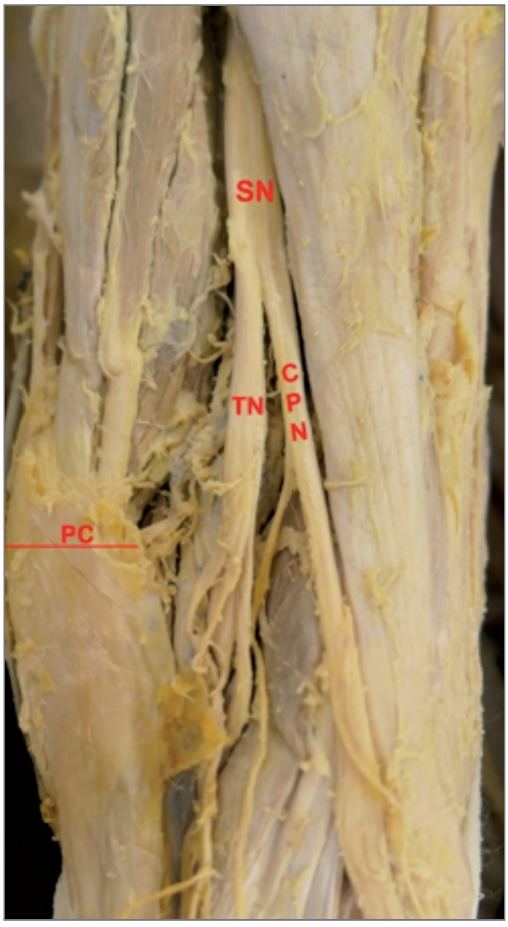

Fig. 1 Division of the sciatic nerve, right side, posterior view. PC – level of the popliteal crease, SN – Sciatic nerve, TN – Tibial nerve, CPN - Common peroneal nerve

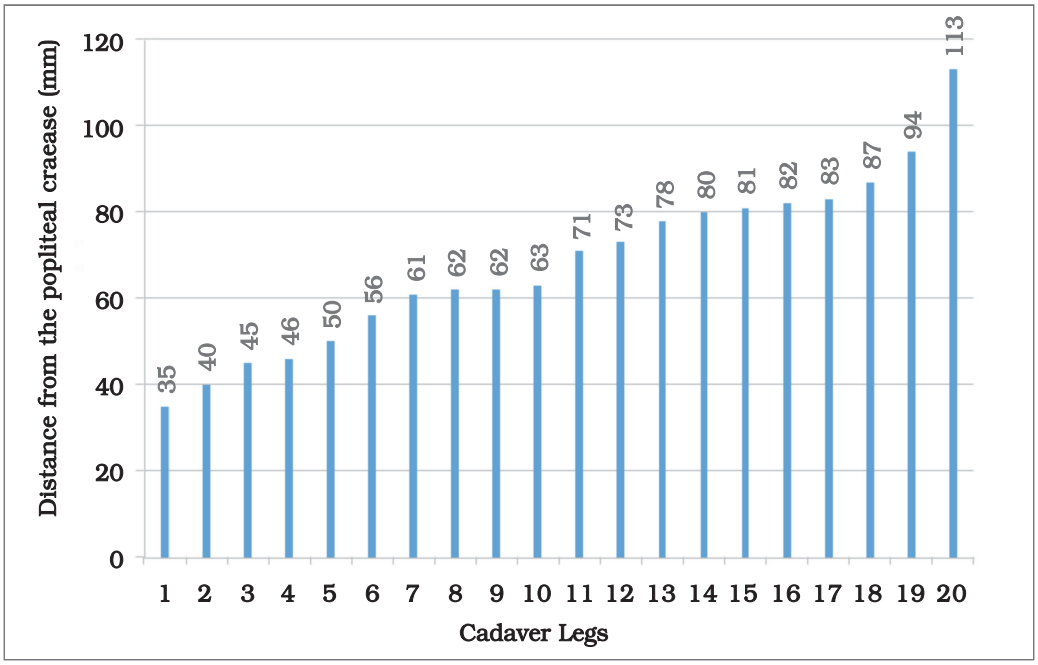

Fig. 2 Division of the sciatic nerve into its component nerves above the popliteal crease.

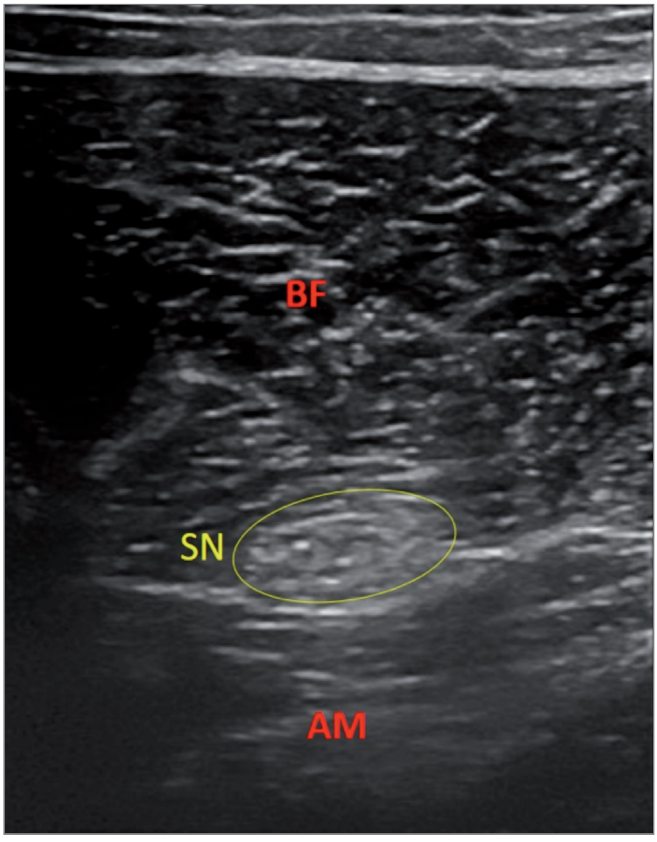

Fig. 3 The mid-femoral sciatic nerve, right side, prone position. BF – Biceps femoris, AM – Adductor magnus, SN – Sciatic nerve

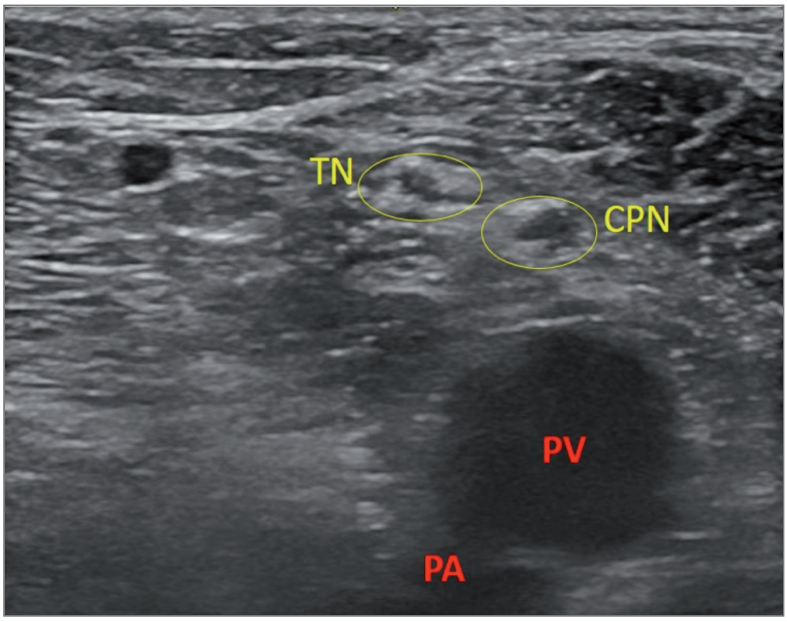

Fig. 4 Division of the sciatic nerve, right side, prone position. TN – Tibial nerve, CPN – Common peroneal nerve, PV – Popliteal vein, PA – Popliteal artery

DISCUSSION

In clinical practice, several techniques for the SN block are used. The majority of methods that have been reported to this date rely on complex superficial anatomical landmarks. Surface anatomical landmarks offer helpful guides for determining the position of the SN, but sometimes these may be difficult to locate. They can differ across patients and their exact localization may be challenging, especially in obese patients. Additionally, it is essential to note that a motor response may not always be elicited by electrical nerve stimulation and success is not always guaranteed (11).

Some authors have reported variants of SN bifurcation into the CPN and TN between the pelvis and PF (12–15). SN anatomy's variations are indispensable to remember, mainly during SN blocks and hip surgery. A higher bifurcation of the SN, where it can split into its terminal branches anywhere in the pelvis or thigh, is a somewhat common occurrence. In the case of the pelvis, the whole nerve or its terminal branches may emerge through the piriformis muscle, above it, or below it (16).

Six anatomic SN variants are described in the classification system of Beaton and Anson. Most often, the undivided SN runs distally to the piriformis muscle (type A). The TN runs inferiorly to the piriformis in type B, while the CPN penetrates the piriformis. The CPN runs in type C superiorly to the piriformis and the TN below piriformis. The undivided SN penetrates the piriformis in type D. The TN penetrates the piriformis, whereas the CPN runs above it in type E. Finally, the undivided SN runs superiorly to the piriformis muscle in type F (17, 18). In addition, some muscle variations in the popliteal region were also described (19, 20). It is requisite to be familiar with the possibility that they may cause difficulty in needle insertion.

Our data from the anatomical study show that the SN splits itself into its two components, CPN and TN, at varied distances from the PC. Variants of the SN may result in an incomplete block because of the local application of anaesthetic close to only one of these components (4). In the realized ultrasound survey, the SN and its division were consistently and reliably identified in all volunteers. The ultrasound image was made optimal after the probe's slow cranially and caudally movement in the posterior mid-femoral region while

preserving the cross-section view until the SN was recognized. Peripheral nerve ultrasound imaging depends on the nerve's shape, spatial orientation, internal architecture, and capability of recognizing nearby structures (10). Our study indicates that the mid-femoral SN and its division can be easily visualized using ultrasound. Ultrasound imaging increases the possibility of achieving a close placement of the tip of the needle to the SN by a successful localization of its division.

CONCLUSION

One of the methods for a lower limb regional anaesthesia is the SN block. SN can be found at various levels from the gluteal region to the PF. It can be reached along the sciatic line which starts from the apex of the PF and ends in the middle of the axis between the ischial tuberosity and the greater trochanter (21). However, anatomical variations of the SN may be the cause of the incomplete block.

In conclusion, our anatomical study's results display the different positions of SN division. In addition, we also describe the ultrasound anatomy and ultrasound appearance of the SN and its major branches.

Acknowledgements

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Ethical approval

Informed consent has been regularly acquired from each and every volunteer included in the manuscript. All cadavers were acquired from the body donation program after signing the informed consent of the donors themselves. No ethical clearance was required as cadavers are used for teaching and research purpose.

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CAPILLAROSCOPY AND ENDOPAT – HELPFUL METHODS FOR THE EARLY ASSESSMENT OF INCREASED CARDIOVASCULAR RISK IN ANOREXIA NERVOSA?

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Abstract

Anorexia nervosa (AN) as a life-threatening eating disorder is linked to a high mortality risk with many deaths attributable to cardiovascular etiology. Cardiovascular complications in AN include structural as well as functional cardiac alterations, hemodynamic changes, and peripheral vascular abnormalities. Despite the fact that peripheral vascular abnormalities are not identified as a major AN complication, several manifestations of peripheral vascular dysregulation including Raynaud's phenomenon and endothelial dysfunction have been described and, therefore, warrant attention. This article briefly summarizes so far findings of microvascular alterations in AN patients and presents easily accessible and non-invasive procedures for a microvascular evaluation such as capillaroscopy and endothelium-related peripheral arterial tone (EndoPAT) which could be involved in the clinical diagnostic process for the earliest identification of an increased risk of later cardiovascular complications.

Keywords: capillaroscopy, endothelial function, peripheral vascular abnormalities, cardiovascular risk, anorexia nervosa

INTRODUCTION

Anorexia nervosa (AN) represents a serious eating disorder commonly associated with other medical complications such as cardiovascular ones. Alterations in the cardiovascular system on the functional (e.g. bradycardia, prolongation of QT interval) and structural (e.g. reduction of left ventricular mass) levels can occur in approximately 75–85% of AN patients (1, 2). Among others commonly reported cardiovascular complications in AN, peripheral vascular abnormalities caused by dysregulation between peripheral vasoconstriction and vasodilatation mechanisms clinically appear with Raynaud´s phenomenon (RP), a distinctive syndrome, characterized by episodic transient spasms of the peripheral vessels leading to digital ischemia associated with digits´ discoloration that may be caused by the reflex vasoconstriction to emotional stress and low temperatures have been described (3–6). In this context, already Luck and Wakeling pointed to elevated cutaneous vaso-responsiveness to cold in anorexic patients (7). Although since then several studies evaluated the relationship between peripheral vascular alterations and AN (for review (5)), future research

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is needed to reveal the precise AN-vascular health association. Moreover, endothelial dysfunction as a key factor in the development of atherosclerotic disease, predicting an increased rate of adverse cardiovascular events, has been described in anorexic patients (8). Therefore, routine monitoring of the cardiovascular health in AN patients should be considered to minimize a cardiovascular disease risk. In this aspect, the evaluation of microcirculation and endothelial function could offer an easy and non-invasive way for the earliest detection of peripheral vascular abnormalities in AN (9, 10).

NAILFOLD CAPILLAROSCOPY (NC)

NC is a non-invasive and easy-to-use imaging technique to evaluate microcirculation and capillary structure in the nailbed area. The main application of NC is the assessment of microvascular impairment in RP with a focus to differentiate between primary and secondary forms of RP (11, 12). The gold standard between capillaroscopic methods to perform nailfold capillaroscopy is the nailfold videocapillaroscopy (NVC) with a 200× magnification, capturing at least two adjacent fields of a linear millimeter (mm) in the middle of the finger (13). It has been shown that the sensitivity to reliably detect capillary abnormalities increases as more fingers were examined with the recommendation of eight-fingers evaluation, however, in the case of time pressure, the two-finger combination (both ring fingers) to detect capillary abnormalities may be used (14). NVC quantitative assessment involves capillaroscopic characteristics such as capillary density, capillary dimension, capillary morphology, and presence/absence of hemorrhages that are standardly evaluated per unit of quantity (i.e. per linear mm). Capillary density as the most reliable capillaroscopic parameter is used for the prediction of the disease progression and treatment monitoring. In NVC qualitative assessment – "scleroderma pattern" – further subgraded as early, active, or late is estimated (as reviewed in (13)). Based on the capillaroscopic characteristics – the image may refer to changes specific to the "scleroderma pattern" as occurring in scleroderma spectrum diseases, or the image is normal or has non-specific abnormalities as occurring in the healthy population, in primary RP or in connective tissue diseases other than systemic sclerosis (13). The normal capillaroscopic pattern is characterized by high skin transparency, absence of morphological abnormalities, uniformity of diameter and distribution of the capillaries, and hairpin capillaries arranged in a parallel fashion to each other. Early scleroderma pattern is defined as enlarged capillaries (diameter >20 μm and <50 μm), few (<4–6/mm) giant capillaries (diameter >50 μm), few microhemorrhages, fragmentation of the blood column with a granular appearance, and lack of capillary loss. Active scleroderma pattern represents more than six giant capillaries/mm, frequent capillary hemorrhages, 20–30% loss of capillaries, disorganization of the capillary architecture; and late scleroderma pattern is characterized by a 50–70% loss of capillaries with large avascular areas, disorganization of the normal capillary array, ramified/bushy capillaries, and neovascularization (9, 13, 15–18). The examination requires a proper preparation of the patient. The patient should have no history of trauma to the distal phalanges (including manicure), so the nail beds show no evidence of an infection and/or wound. The patient should not have artificial nails or nail polish. Moreover, the patient should be placed at least 15 minutes before the examination at a standard room temperature (22–25°C) to allow the nail fold capillary network to adapt (19).

It is important to note that there is an evidence of the association between nailfold capillaroscopic morphology and palmar digital arteries´ morphology and blood flow findings indicating that the microvascular damage and its progression is linked to macrovascular disease contributing to the increased risk of cardiovascular events (20). Moreover, the follow-up study has reported the association between RP and increased cardiovascular mortality indicating that RP can represent a precursor of undiagnosed vascular disease (21). Thus, present symptoms of microvascular abnormalities deserve attention in anorexic patients.

EVALUATION OF THE ENDOTHELIAL FUNCTION (EndoPAT)

Assessment of the flow-mediated arteries´ vasodilation dependent on the proper endothelial function represents an entrenched measure of vascular health (22). Healthy endothelium as a monolayer of endothelial cells covering the vascular beds´ lumen produces a wide range of factors as a response to various signals to regulate predominantly the vascular tone *via* the release of vasoactive molecules constricting or relaxing the vessels playing a key role in the maintaining the balance of oxygen supply to tissues and metabolic demand. Healthy endothelium also regulates the proliferation of smooth muscle cells or cellular adhesion (23, 24). Contrary, endothelial dysfunction is characterized by an imbalance between vasodilators and vasoconstrictors leading to an impairment of endothelium-dependent vasodilation, i.e. vessels´ inability to adequately dilate in response to the stimulus. Moreover, endothelial dysfunction also comprises the activation of pro-inflammatory and pro-coagulatory *milieu.* Endothelial dysfunction thus represents a major contributor to atherosclerosis and a well-known predictor of an increased cardiovascular risk (25–27). Endothelial (dys)function can be assessed by numerous methods including the EndoPAT device which records the arterial pulse volume changes through the plethysmographic probes placed on the index fingers of each hand and transforms them to a peripheral arterial tone signal. A measure of endothelial function *via* changes in the vascular tone mediated by the endothelium is elicited by creating a hyperemic response after occlusion of the blood flow through the brachial artery with a cuff inflated to supra-systolic values for 5 minutes on the non-dominant hand followed by a consequent blood pressure cuff deflation. The recorded values are normalized to measures recorded from the contra-lateral arm used to control the concomitant non-endothelium dependent changes in peripheral arterial tone (e.g. fluctuations in the sympathetic nervous system activity) (25, 28–30). The obtained data are automatically analyzed by the system with the calculation of an endothelial function index (i.e. Reactive Hyperemia Index – RHI) defined as the ratio between average pre-occlusion baseline pulse wave amplitude and post-occlusion pulse wave amplitude measured during the one-minute period after 90 seconds of reactive hyperemia beginning, with RHI value less than 1.67 considered as abnormal (31). Limitations for the examination include digits´ deformities which preclude adequate signal acquisition, medical conditions prohibiting blood flow occlusion in both arms, and the use of short-acting nitroglycerine (31).

PERIPHERAL VASCULAR ALTERATIONS IN ANOREXIA NERVOSA

Regarding the synergistic relationship of AN and RP on microvascular impairment, De Martinis et al. (9) revealed an early scleroderma pattern in 59% of patients suffering from both (AN+RP) and in 55% of RP patients with AN history. These findings outline the detection of capillaroscopic abnormalities in AN patients/individuals formerly suffering from AN with concomitant RP (9). Regarding AN and RP during adolescence, Kasap-Cuceoglu et al. revealed minor and major capillaroscopic changes including enlarged capillaries, mild tortuosity, microhemorrhages, capillary ramifications, capillary disorganization, and capillary loss in AN adolescents. Moreover, there were only minor capillaroscopic findings including mild capillary dilation and tortuosity in adolescents with RP (32). It is important to note that an association between capillaroscopic microvascular abnormalities and peripheral endothelial dysfunction was found (33, 34). In the view of endothelial dysfunction assessed by EndoPAT in anorexia nervosa, Suntharos et al. reported no significant differences in RHI between anorexic patients and the control group (10), while Springall et al. revealed a reduced endothelial function in young adult AN patients who formerly suffered from AN during adolescence (35). Moreover, Palova et al. reported a decreased vasodilatation in AN patients compared to the healthy subjects using the evaluation of flow-mediated dilatation of the brachial artery (8). Based on these above-mentioned findings, future research in the AN-microvascular health association and its contribution to cardiovascular complications is needed. Potential peripheral vascular abnormalities associated with AN are summarized in Figure 1.

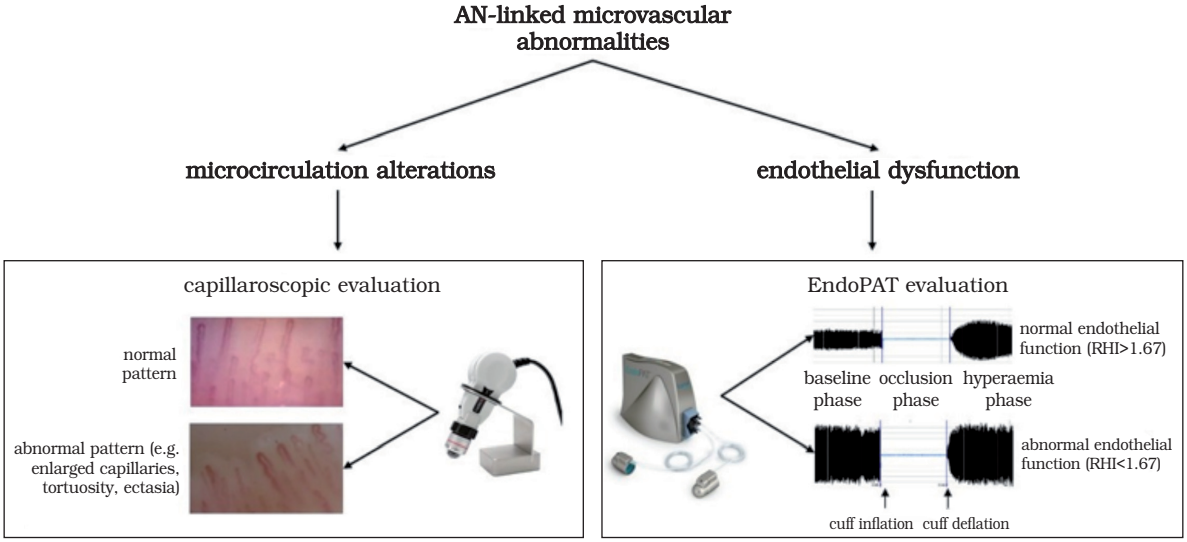

Fig. 1. Evaluation of peripheral vascular abnormalities which can be associated with anorexia nervosa (AN). RHI – Reactive Hyperemia Index

CONCLUSION

The nailfold capillaroscopy and EndoPAT can be considered promising non-invasive methods for an early detection of AN-linked microvascular damage which, in combination with additional methods, may represent important diagnostic tools to detect the earliest peripheral vascular dysfunctions in anorexic patients. We believe that a detailed non-invasive assessment of AN-linked vascular abnormalities could contribute to a better understanding of pathophysiological mechanisms leading to a higher cardiovascular risk in anorexia nervosa, improving thus an early diagnosis, personalized prevention, and therapy of AN-linked cardiovascular complications.

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PLATELET miRNA EXPRESSION IN PATIENTS WITH STICKY PLATELET SYNDROME

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Abstract

Sticky platelet syndrome (SPS) is a disorder with familial occurrence and autosomal dominant trait characterized by platelet hyperaggregability in response to a low concentration of adenosine diphosphate (ADP) and/or epinephrine (EPI). The etiology of SPS may be associated with platelet microRNAs (miRNAs), which are considered as potential biomarkers of platelet function and antiplatelet therapy. We were monitoring the expression of platelet miRNAs in patients with laboratory diagnosed SPS and healthy controls. We have found a statistically significant increased expression of both miR-423-5p and miR-338-3p as well as a statistically significant decreased expression of miR-425-5p between the group of patients with diagnosed SPS type II and the group of healthy controls, which seems to be an interesting issue for a further research.

Key words: sticky platelet syndrome, miRNA, expression

INTRODUCTION

Sticky platelet syndrome is a thrombophilic thrombocytopathy defined as hyperaggregability in response to a low concentration of adenosine diphosphate (ADP) and/or epinephrine (EPI), while aggregability after other reagents is normal. Diagnostics is based on laboratory results of aggregation testing. According to the aggregation pattern, three types of the syndrome can be identified (Type I = hyperresponse after both inducers, Type II = hyperresponse after EPI alone, Type III = hyperresponse after ADP alone). Clinical manifestation is mostly associated with venous or arterial thrombosis. The first thrombotic event usually occurs before 40 years of age and without prominent acquired risk factors. Direct genetic background of this inherited thrombophilic disorder is not sufficiently known, but the genetic changes of platelet membrane receptors play a possible role in platelet activation and aggregation (1, 2).

An interesting issue for the research of SPS etiology seems to be platelet miRNAs. MiRNAs are small, non-coding RNAs, which are able to regulate cellular functions by specific gene modifications and they have been shown to modify the expression of platelet proteins. They are predicted to modulate many targets and they are involved in regulating various cellular processes. Circulating miRNAs can be used as diagnostic and prognostic biomarkers of vascular thrombosis affecting platelet reactivity as well as novel therapeutic targets. Various clinical studies have shown associations between circulating miRNA levels and platelet reactivity pathways or the recurring cardiovascular events (3,4,5).

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AIM

Monitoring the expression of chosen platelet miRNAs in patients with laboratory diagnosed SPS and considering their possible role in pathogenesis of SPS.

MATERIAL AND METHODS

Following population presents 40 samples of isolated platelets, 18 patients with SPS type I, median 32 years (5–52), 10 patients with SPS type II, median 30 years (13–45), and 12 healthy controls, median 31 years (22–54). For the analysis of samples we used light transmission aggregometry (LTA, Agg-RAM, Helena Laboratories, USA) established by Mammen (6) and Bick (7). The platelet function was evaluated by testing the aggregation in response to 2 low concentrations ADP and EPI (0.58 and 1.17 mmol/L). SPS I was confirmed by hyperaggregation in response to at least 1 low concentration of ADP and EPI. SPS II was confirmed by hyperaggregation in response to 2 low concentrations of EPI.

For platelet separation we used magnetic separation system MidiMACS™ Separator (MiltenyiBiotec, Zürich, Switzerland), together with superparamagnetic plastic particles (MicroBeads – MiltenyiBiotec GmbH, Bergisch Gladbach, Germany), which are conjugated with CD45 and CD235a antibodies for the removal of white blood cells and granulocytes from the samples. The CD45 MicroBeads were used for a positive selection or depletion of leukocytes from the peripheral blood and the CD235a MicroBeads were used for a positive selection or depletion of human erythroid cells. Unlabelled cells passed through the separator while magnetically labelled cells were retained within the separator. The number of cells was determined by the blood cell analyser (8).

MiRNA was isolated by High Pure miRNA Isolation Kit (Roche, Germany). For the measurement of chosen platelet miRNAs expression *(hsa-miR-221-3p, hsa-miR-33a-5p, hsa-let-7b-5p, hsa-let-7i-5p, hsa-miR-423-5p, hsa-miR-338-3p, hsa-miR-148a-3p, hsa-miR-22-3p, hsa-miR-425-3p, hsa-miR-29b-3p, hsa-miR-326, hsa-miR-18a-5p, hsa-miR-148b-3p, hsa-miR-331-3p, UniSP3, UniSp6)* we used a real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) with miRCURY® LNA® miRNA PCR Assays and PCR panels (Qiagen, Germany) exosome isolation kit. For normalization we used a panel for platelet miRNA consisting of *miR-148b, miR-151-3p, miR-18a, miR-28-5p, miR-29c, miR-331-3p*. We were monitoring the fold change DELTA, which is a fold gene miRNA expression compared to the control. The average value of the control equals 1. If the fold change DELTA is <1, miRNA expression is reduced and if the fold change is >1, the expression is increased.

All data we got from qRT-PCR were processed in QIAGEN GeneGlobe Data analysis center and statistical tests were performed in Microsoft Excel spreadsheet. The results with p-value < 0.05 were considered statistically significant (9).

RESULTS

We were monitoring the platelet miRNAs expression in patients with diagnosed SPS and healthy controls. We surveyed the statistically significant increased expression of both miR-423-5p (fold change 1.33; p=0.05) and miR-338-3p (fold change 1.32; p=0.05) as well as the statistically significant decreased expression of miR-425-5p (fold change 0.69; p=0.01) between the group of patients with SPS type II and the group of healthy controls (Tab.1).

The expression of miR-29b-3p in patients with SPS type II was significantly decreased, but this change was not statistically significant (p=0.38). In the group of patients with SPS type I we have not found any statistically significant changes of the platelet miRNAs expression .

Table 1 Statistically significant changes of platelet miRNAs expression

miRNA (mature ID)	Fold change DELTA		p-value		Comment
	SPS1	SPS2	SPS1	SPS2	
hsa-miR-423-5p	1.13	**1.33**	0.28	**0.05**	increased expression in SPS 2
hsa-miR-338-3p	1.07	**1.32**	0.33	**0.05**	increased expression in SPS 2
hsa-miR-425-3p	0.92	**0.69**	0.55	**0.01**	decreased expression in SPS 2
hsa-miR-29b-3p	0.81	0.49	0.89	0.38	

Abbr.: n – number, miRNA – microRNA, SPS – sticky platelet syndrome

DISCUSSION

The pathogenesis of sticky platelet syndrome remains unknown and the diagnosis is based on the laboratory results and clinical manifestation. Platelet microRNA may have a potential role as a biomarker of platelet hyperaggregability (9).

MiRNAs are non-coding RNAs which are able to modify the expression of platelet proteins influencing platelet activity. Circulating miRNAs have been suggested as diagnostic and prognostic biomarkers, which can be also used as therapeutic targets. MiRNAs modify the platelet proteins expression by targeting mRNAs, which alters their biochemical pathways. Some biochemical pathways are associated with drug response, which means they can impact on efficiency of antiplatelet therapy responsiveness. Platelet-related miRNAs have been suggested also as a biomarker for the assessment of antiplatelet therapy efficiency by various clinical studies. Antiplatelet therapy is recommended as a lifelong therapy for patients with SPS. Molecular mechanisms of platelet pathways are important for further studies, because a large number of patients requires antiplatelet treatment (3,10,11).

MiRNAs are rising to be the possible biomarkers for both cardiovascular diseases and cancer. They play a role in regulating the expression of many proteins involved in hemostasis and cancer and they may help to understand and predict the risk of venous thromboembolism (VTE). The increased expression of miR-423-5p have been shown to be a diagnostic biomarker of deep vein thrombosis (DVT). Its potential aim is mRNA in genes VEGF and eNOS. Unfortunately most of the studies used only small sample sizes and lacked external validation (12).

Recently we were monitoring the changes in expression of miR-96-5p, miR-126-3p, and miR-223-3p in platelets of 45 patients with diagnosed SPS and 30 healthy controls. We have not found any correlation between these 3 chosen miRNAs and the phenotype of SPS, but interestingly we have found a significantly increased expression of miRNA-96-5p in platelets of SPS patients with pregnancy complications ($p<0,01$) (9).

Our present study was monitoring the expression of chosen miRNAs in patients with SPS. Current results showed a statistically significant increased expression of both miR-423-5p and miR-338-3p as well as a statistically significant decreased expression of miR-425-5p between the group of patients with diagnosed SPS type II and the group of healthy controls. The expression of these 3 miRNAs can be associated with the pathogenesis of SPS and we consider it as an interesting issue for further clinical studies in larger population.

However, comparing the results of different studies has several limitations. The expression of miRNAs is being measured in different types of biological samples (platelet poor plasma, platelet rich plasma or isolated platelets) and different laboratory protocols are used, some

types of miRNAs expressed by platelets can be also released by other cells, platelet reactivity is measured by various incomparable methods and the results of studies depend on genetic variability of populations living in the different geographical regions. In future the standardization of measurement is needed to establish many practical aspects and to get comparable results (3).

CONCLUSION

MiRNAs play an important role in platelet function and reactivity pathways of platelets. Some of them can aim on mRNA coding proteins, which are associated with aggregation, thus they can regulate their expression. According to our results the increased expression of platelet miR-423-5p and miR-338-3p as well as the decreased expression of miR-425-5p seem to be an interesting issue for a further research and testing in larger group of patients with SPS.

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DELIVERY MODE AFFECTS THE SYMPATHETIC NERVOUS SYSTEM IN HEALTHY TERM NEWBORNS

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Abstract

Background: Spontaneous delivery represents an important way triggering the physiological mechanisms essential for a proper postnatal adaptation of a newborn. Autonomic nervous system (ANS) plays a crucial role in this process. There is insufficient data concerning the impact of different delivery mode on ANS in newborns. Therefore, we aimed to study the effect of delivery mode on sympathetic nervous system (SNS) in healthy term newborns measured by electrodermal activity (EDA).

Material & Methods: The study conducted 50 healthy full – term newborns divided into two groups: the spontaneous delivery group (SD, n=27) and the caesarean section group (CS, n=23). EDA parameters (SCL – tonic level of skin conductance; NS.SCRs – non – specific phasic responses of skin conductance) were evaluated during three measurements: 2nd (M1), 24th (M2), and 72nd (M3) hours of life.

Results: SCL significantly decreased during the first day of life (M1 vs. M2 – $p < 0.001$). The lowest NS.SCRs values were demonstrated in M1 in both groups with significant differences in CS group between measurements – M1 vs. M2 ($p=0.001$) and M1 vs. M3 ($p=0.005$). However, a significantly lower NS.SCRs was found in CS group ($p=0.01$) early after birth (M1).

Conclusion: Our findings revealed a reduced NS.SCRs indicating potential decreased „arousal" in CS group – it seems that the attenuated „arousal" could reflect the absence of physiological labor mechanisms as well as the effect of anesthesia leading to discrete early functional abnormalities in CS group. Further research is needed to validate these findings.

Key words: newborn, delivery mode, sympathetic nervous system, electrodermal activity

INTRODUCTION

Spontaneous delivery (SD) is a natural way of human birth. From the physiological aspect, SD represents a crucial stress trigger, which is the substantial premise for a normal postnatal adaptation of a newborn. Several mechanisms participate on the triggering of perinatal stress – the onset of labor, change of environment (in utero to ex utero) with the mechanical (e.g. pressure of the birth canal) and temperature (and many other) stimuli affecting the neonatal organism during peripartal period (1). All these factors result into the activation of sympathetic nervous system (SNS) together with an increased

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production of catecholamine and cortisol in neonate (2). This is very important for the extrauterine adaptation of the respiratory and circulatory systems, thermogenesis, and energy metabolism (1). The labor without associated neonatal stress (i.e. elective caesarean section) is a potential factor which interferes in the process of postnatal adaptation (3).

The autonomic nervous system (ANS), consisting of SNS and parasympathetic nervous system (PNS), is extremely important for postnatal adaptation in newborns. More specifically, SNS enables brisk adaptation to postnatal life (2). From the developmental aspect, SNS is characterized by steady maturation during the pregnancy and requires at least 37 gestational weeks to reach accurate functioning (4). Maturation of ANS also continues after birth with PNS predominance in term neonates (5).

Electrodermal activity (EDA) also known as skin conductance (SC) represents a useful parameter reflecting the changes exclusively in SNS (6). The measurement of EDA reflects the changes of the skin electrical conductivity due to the activity of eccrine sweat glands. This phenomenon is under the pure control of sympathetic cholinergic nervous system (7). Peripheral SNS together with sweat glands located in the palms and plants are developed in the second trimester of pregnancy and continue to mature (8). In term newborns, fully developed functional emotional sweating was reported (9). From this perspective, EDA method represents an optimal tool for the assessment of SNS function in term newborns. In addition, EDA measurement is a safe, non – invasive, easy to handle method only little influenced by other factors (e.g. circulatory changes).

Understanding of normal early postnatal ANS function in full – term healthy newborns may provide a useful reference for studies of high – risk neonates and may have a prognostic value. Therefore, it is important to elucidate how different delivery modes influence the measures of ANS function in low – risk term newborns (10). To the best of our knowledge, there are no studies concerning the influence of delivery mode on SNS function and maturation measured by EDA within the first postnatal days in healthy term neonates. Therefore, we aimed to study potential changes in SNS indexed by EDA in early postnatal adaptation period in newborns depending on the delivery mode.

MATERIALS AND METHODS

Subjects:

Our study was conducted from January 2019 to February 2020 at the Clinic of Neonatology of the University Hospital and Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava. The study included 50 healthy eutrophic full – term newborns with physiological immediate postnatal adaptation (Apgar score ≥ 8 points in the 1st minute and ≥ 9 in the 5th and 10th minute) who were divided into 2 groups according to the delivery mode: the spontaneous delivery (SD) group and the caesarean section (CS) group. All women who underwent caesarean section were in general anesthesia (a combination of intravenous thiopental and the inhaled sevofluran). Only newborns with indications of elective caesarean section were included (breech presentation, repeat caesarean section, primary maternal indication for caesarean section). SD group consisted of babies without the need for instrumental delivery (i.e., forceps and vacuum extractor).

Exclusion criteria involved all conditions that are proven or very likely associated with abnormal ANS function: pathological gravidity (preterm delivery, intrauterine growth restriction, etc.), maternal systemic disease (gestational or another types of diabetes), smoking during pregnancy, congenital anomaly of the neonate, signs of perinatal infection, metabolic disorders: hypoglycaemia and hyperbilirubinemia requiring phototherapy during hospitalization.

Measurement and data analysis:

The examination was performed during the day (7 am – 12 am) and during sleep or quiet alert state in the supine position under standard conditions with the minimization of internal and external stimuli. Two silver – silver chloride bipolar electrodes with a sampling rate of 256 Hz placed on the sole of the neonate were used for the continuous EDA recording (FlexComp Infinity Biofeedback, Thought Technology Ltd., Canada) according to the recommendation of EDA biosignal measurements (11). The examination protocol included three EDA measurements that were performed 2 hours (measurement 1 – M1), 24 hours (measurement 2 – M2), and 72 hours (measurement 3 – M3) after birth. After the stabilization of the subjects (approximately 10 minutes – adaptation interval) the measurement of EDA was initiated (6 minutes records). EDA raw records were checked and the artefacts were removed for the final data analysis. The tonic EDA component was extracted by the 10^{th} order low – pass finite impulse response filter (12). The following EDA parameters were analyzed: 1) ***skin conductance level*** (SCL, microSiemens (μS)) – an index of tonic level of skin electrical conductivity; 2) ***non – specific skin conductance responses*** (NS.SCRs) – reflecting momentary *"arousal"* with fixed threshold (0.05 μS – evaluated as a rate of spontaneous skin conductance response waves without external stimuli) (13).

Statistical analysis:

Jamovi version 1.6.9 (Sydney, Australia) was used for the statistical analysis and visualization of the measured data. The distribution and normality of the data was assessed using a violin plot and the Shapiro – Wilk normality test. Because of non – normal data distribution, the non – parametric ANOVA (Friedman test with post hoc Durbin – Conover test) was used. A value of $p < 0.05$ (two – tailed) was considered statistically significant.

Ethical approval:

This paper presents the preliminary data of our prospective observational study „Assessment of Autonomic Nervous Regulation During Postnatal Period in Newborns" registered in Clinical Trials (ID NCT03830424). The clinical study was approved by the Ethics Committee of the Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava (EK 69/2018). All procedures performed in the study were in accordance with the 2000 Helsinki declaration of the World Medical Association. The subjects were included after meeting the inclusion criteria and with an approval by their parents after signing the informed consent.

RESULTS

Our set of subjects consists of 50 newborns (28 girls, mean gestational age 39 ± 0.21 weeks, mean birthweight 3362 ± 79 grams). Baseline characteristics of subject groups according to delivery mode is presented in **Table 1**.

Intragroup comparison:

Non – parametric Friedman test showed a significant effect of delivery mode for the parameters NS.SCRs ($F[1]=6.66$; $p=0.01$) and a mixed effect of mode of delivery and phase for the parameters SCL ($F[5]=42.0$; $p<0.001$), NS.SCRs ($F[5]=25.9$; $p<0.001$). Post – hoc analysis revealed a significantly higher SCL in M1 vs M2 (SD: $p<0.001$; CS: $p<0.001$) and M1 vs M3 (SD: $p=0.003$; CS: $p=0.028$) in both groups. However, a significant difference in NS.SCRs was observed only in CS: significantly lower values in M1 vs M2 ($p=0.001$) and M1 vs M3 ($p=0.005$) (**Fig 1**). There was no difference in the remaining parameters.

Intergroup comparison:

The statistical analysis revealed a significantly lower NS.SCRs in neonates born by CS vs SD in M1 ($p=0.01$). (**Table 2**). No significant differences were revealed in the remaining parameters.

Table 1 Baseline characteristics of subject groups

DELIVERY MODE			
	SD	CS	p
N (girls)	27 (17)	23 (12)	–
GA (weeks)	39.4 ± 0.23	38.4 ± 0.34	***0.017***
BW (g)	3383 ± 113	3335 ± 110	0.685

BW = birthweight, CS = caesarean section, GA = gestational age, N = number of subjects, SD = spontaneous delivery

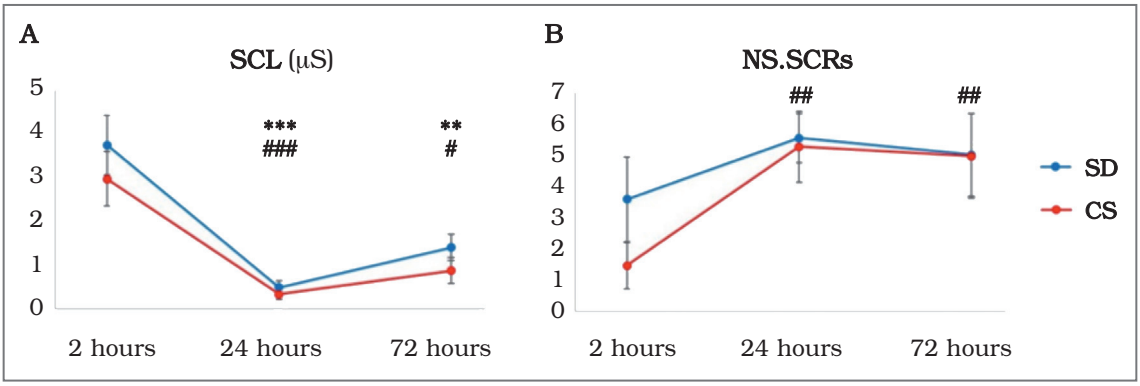

Fig. 1 Changes of EDA parameters within first three postnatal days according to the delivery mode. A) SCL – skin conductance level; B) NS.SCRs – nonspecific skin conductance responses. CS = caesarean section; SD = spontaneous delivery, μS = microsiemens. Values are present as a mean ± standard error of the mean (SEM). Symbols: * = significant difference between the measurements in SD group; # = significant difference between the measurements in CS group; symbols above „24 hours" represent the difference between M1 and M2; symbols above „72 hours" represent the difference between M1 and M3; $^{*,\#}$ $p < 0.05$; $^{**,\#\#}$ $p < 0.01$; $^{***,\#\#\#}$ $p < 0.001$.

Table 2 Comparison of EDA parameters between groups

	M1 [a]		M2 [b]		M3 [c]		p [a]	p [b]	p [c]
	SD (n=27)	CS (n=23)	SD (n=27)	CS (n=23)	SD (n=27)	CS (n=23)			
SCL (μS)	3.74±0.68	2.96±0.62	0.49±0.15	0.33±0.12	1.40±0.30	0.87±0.30	0.461	0.540	0.964
NS.SCRs (/min)	3.58±1.35	1.46±0.74	5.54±0.79	5.26±1.13	5.00±1.33	4.96±1.35	***0.010***	0.457	0.597

SCL – skin conductance level; NS.SCRs – nonspecific skin conductance responses. CS = caesarean section; SD = spontaneous delivery; μS = microsiemens; p = statistical significance; n = number of subjects; M1 = 1st measurement; M2 = 2nd measurement; M1 = 3rd measurement

DISCUSSION

Our study investigated the potential effect of delivery mode on SNS activity and maturation during early postnatal life in healthy term neonates evaluated by EDA analysis. The major findings of this study are following: 1) significantly higher values of SCL were observed early after birth (M1) in all newborns followed by a significant decrease during the following measurements (M2 and M3) reflecting a post – delivery stress – related sympathetic withdrawal after postnatal adaptation; 2) NS.SCRs representing the *„arousal"* phenomenon was lower in all subjects early after birth (M1) compared to the following measurements (M2 and M3). The increase within 24 hours of life may reflect changes related to an increased sensory processing and receptor activation related to the adaptation to the environment; 3) NS.SCRs was significantly lower in CS group reflecting the effect of delivery mechanism and peripartal general anesthesia administered to the mothers undergoing CS. Based on our results we suggest that EDA measurement may represents a sensitive tool to determine the developmental and maturational changes in SNS during the earliest postnatal life. Several mechanisms are supposed:

During the peripartal period, the SNS outflow from central ANS structures increases and together with neuroendocrine support (e.g. catecholamines) enables brisk postnatal adaptation (2). After birth, SNS constantly continues to develop. Regarding the development and normal functioning of palmar and plantar sweat glands, Harpin and Rutter (9) reported functional emotional sweating in term neonates. Our study showed the highest values of SCL in all newborns 2 hours after birth supporting the functionality of eccrine sweat glands in term neonates immediately after delivery. According to our results, SNS activity decreased within the first 24 hours of life in both groups reflecting the depression of acute stress adaptation process triggered by the delivery. Therefore, EDA is a sensitive marker which is able to detect these subtle changes in sympathetic regulatory mechanisms.

Further, the elevation of NS.SCRs within the first postnatal day in all newborns indicates a physiological excitation of central nervous system. NS.SCRs as a discrete fluctuation of skin conductance around the baseline arise spontaneously, without the influence of an external stimuli and reflect states of increased "arousal" (14). The increase of NS.SCRs within the first postnatal day may reflect changes related to an increased processing of perceptions and the activation of receptors in the context of adaptation to the extrauterine environment.

Lower NS.SCRs values in CS group during the immediate postnatal adaptation could be explained by the absence of physiological delivery mechanisms associated with stress response represented by the activation of SNS and stress hormones (10). Another importnant factor contributing to the lower SNS activity is the general anesthesia of the mother during CS – an sympatholytic effect was described for sevoflurane and thiopental (15, 16) – both drugs were used in our study. However, this effect seems to be temporary.

The precise understanding of SNS development in the earliest postnatal life is limited – there are no studies in the literature focusing on EDA measurements with regard to the mode of delivery in newborns so far. Therefore, our findings can provide novel insights into the SNS regulatory mechanisms immediately after birth and during the earliest postnatal life.

CONCLUSION

Our study evaluated the effect of delivery mode on SNS measured by EDA in healthy term newborns within the first three postnatal days. The results revealed a lower *"arousal"* in CS group early after birth reflecting the absence of physiological labor mechanisms and the effect of general anesthesia on SNS leading to discrete early functional abnormalities in babies born by CS. Further research is required to confirm this finding.

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FRACTURED EXHALED NITRIC OXIDE AND BIOLOGIC THERAPIES FOR PAEDIATRIC ASTHMA

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Abstract

Bronchial asthma is the most frequently diagnosed chronic respiratory disease in children. Treatment approaches should aim to achieve the disease control, reduce limiting symptoms, and improve the quality of life. Routine treatment of patients with asthma relies on assessment of symptoms and spirometry results. These diagnostic and therapeutic strategies do not consider the level of inflammation in the airways as a fundamental pathognomonic feature of the disease. The use of biomarkers is increasing in the context of efforts to better understand individual asthma pathways (asthma endotyping), with the potential for personalized treatment with innovative biologics. Elevated levels of exhaled nitric oxide (FENO) represent an indirect marker of T2 inflammation in airways. FENO is one of the few biomarkers that have been applied in routine clinical practice. High levels predict a good therapeutic response to treatment with corticosteroids and selected biologics (Omalizumab, Dupilumab, Mepolizumab, Tezepelumab), or an increased risk of asthma exacerbation. The aim of this review is to evaluate the advantages, disadvantages, and potential applications of this test in relation to new treatment options using biologics for asthma.

Keywords: asthma, biologics for asthma, biomarkers, children, exhaled nitric oxide, T2 type inflammation

INTRODUCTION

Bronchial asthma (AB) is the most common chronic respiratory disease in children (1). The prevalence of asthma is constantly increasing, resulting in an increased economic impact on its diagnosis and treatment, particularly in developed countries. According to an epidemiological analysis by the US Center for Disease Control and Prevention, the prevalence of asthma in the United States in 2017 was 7.9%, with a higher prevalence in children (8.4%) than in adults (7.7%) (2).

The main pathognomonic entity of asthma is chronic inflammation of the lower airways associated with reversible bronchial hyper-responsiveness and structural changes in the tracheobronchial tree (3). Recurrent bronchial obstruction is linked with various clinical symptoms, including anamnestically reported dyspnea, wheezing, chest tightness, limitation of physical activity, and chronic cough. It is a heterogeneous clinical syndrome with a specific etiopathogenesis and variable clinical manifestations. Considering this, great efforts have been made to understand the individual pathophysiological processes to classify this disease into unique endotypes and phenotypes (4). Asthma treatment focuses on monitoring the activity and severity of the disease (5).

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The treatment of an asthmatic patient that relies solely on the control of reported symptoms may not be successful over a long period. Achieving control over the limiting symptoms does not reflect the risk of predicting future asthma flares (6). In young children, the diagnosis of asthma is particularly challenging. The diagnostic approach in children is often complicated by inability to perform some tests used in older individuals (7, 8). Common methods for asthma diagnosis (questionnaire methods, peak expiratory flow, spirometry, and bronchial challenge tests) do not assess the level of airway inflammation (9, 10).

BRONCHIAL ASTHMA AND EOSINOPHILIC INFLAMMATION

AB is a multifactorial disease with heterogenous patterns of airway inflammation. A combination of heterogeneous extrinsic and intrinsic factors determines the development and persistence of chronic inflammation of the bronchial wall in genetically predisposed individuals. In the new era of evidence-based personalized medicine, efforts are being made to identify measurable pathomechanisms that target a specific group of affected patients (11). This approach enables access to targeted, effective, and innovative treatment. By blocking specific causal pathways, we can achieve better disease control, reduce limiting symptoms, and decrease the use of conventional therapy (12). Finally, attention should be paid to the health of individual patients and their significant improvements in quality of life.

The T2 endotype of asthma is predominant in childhood, in contrast to adults. This endotype is characterized by inflammation with a predominance of eosinophils in an allergic or non-allergic immune microenvironment (13). Considering this, we do not have to prove laboratory sensitization to inhalant allergens in a proportion of pediatric patients. The Th2 subpopulation of lymphocytes and natural lymphoid cell type 2 (ILC2) are crucial for the pathogenesis of the T2 endotype (14). Damage to the airway epithelium due to exogenous factors (allergens, viruses, and pollutants) leads to a loss of functional and structural integrity of the bronchial epithelial barrier (15). The activation of innate and adaptive immune mechanisms causes to the production of epithelial cytokines called alarmins (IL-25, IL-33, and TSLP-thymic stromal lymphopoietin). TSLP is a cytokine produced by the epithelial cells in the skin, gut, lungs, and thymus. It is a member of the interleukin-7 (IL-7) family of cytokines. TSLP binds to a receptor called TSLP receptor (TSLPR), which is a heterodimer of the IL-7 receptor alpha chain. TSLP and other members of the alarmin group directly activate Th2 and ILC2 cells to produce a characteristic cytokine spectrum (IL-4, IL-5, and IL-13) with pleiotropic functions (16, 17). This results in typical functional and anatomical changes in the affected bronchi, such as goblet cell hyperplasia, smooth muscle hypertrophy, neovascularization, mucosal tissue fibrosis, and bronchial hyper-responsiveness (18). IL-13 is the main effector cytokine of T2 inflammation (Fig.1).

Eosinophils are essential components of the inflammatory processes. They are granulocytes that originate in the bone marrow from a myeloid precursor. They also play an important role in maintaining the internal environment of the body within physiological limits (19). For example, resident eosinophils are involved in the regulation of glucose metabolism and adipose tissue (20). Secretory granules located in the cytoplasm of these cells contain many cytotoxic mediators and vasoactive substances that participate in defense against viral and parasitic pathogens (21–23). In contrast, pro-inflammatory eosinophils are responsible for a variety of eosinophil-derived diseases that can affect almost any organ system. Eosinophils help activate bronchial fibroblasts to produce pro-fibrotic factors by secreting various mediators. Therefore, they participate in bronchial remodelling and the thinning of the basement membrane of the epithelial layer. Furthermore, synthesized pro-inflammatory mediators promote bronchial smooth muscle contractility and inhibit relaxant stimuli (24, 25). The IL-5 produced by immunocompetent cells is crucial for the growth, maturation, and migration of the pro-inflammatory phenotype of eosinophils to the site of the inflammation (26). Inflammation-activated epithelial cells also synthesize special chemokines called class

1 (CCL11) and class 2 (CCL24) eotaxins. Eotaxins act on the endocrine system of eosinophils after binding to a specific receptor on their cell surface (predominantly through CCR3). The initiation of connected molecular interactions triggers signalling pathways that result in their migration to the site of the inflammation. Therefore, eotaxins regulate the bone marrow as potent chemoattractants (27,28). The main pathways that result in the development of T2 type asthma are shown in Fig. 2.

Patients with the T2 endotype often demonstrate a good therapeutic response to inhaled corticosteroids and a better response to bronchodilator therapy with beta-adrenergic receptor agonists (13). They also show a favourable response to properly selected monoclonal antibodies that block cytokines from T2 inflammation. Elevated peripheral blood eosinophils, increased number of eosinophils in bronchoalveolar lavage fluid, and total IgE antibodies are markers of this asthma endotype (29). FENO belongs to this category. Typically, during endotyping and phenotyping of asthma, the focus is on the number of eosinophils in the peripheral blood, induced sputum, or bronchoalveolar lavage fluid. The paediatric population faces significant limitations in the collection of biological materials owing to their specificities. In exceptional cases, induced sputum is examined. It requires active cooperation (troublesome in children) and the inhalation of a hypertonic solution (risk of bronchospasm). The evaluation of bronchoalveolar lavage requires flexible invasive bronchoscopy associated with general anaesthesia. These limitations render these tests suitable for more serious and complicated diagnostic cases. Peripheral blood eosinophil counts are widely available as a part of differential blood cell counts. Interestingly, peripheral eosinophilia does not correlate with the number of eosinophils in the tissues (30).

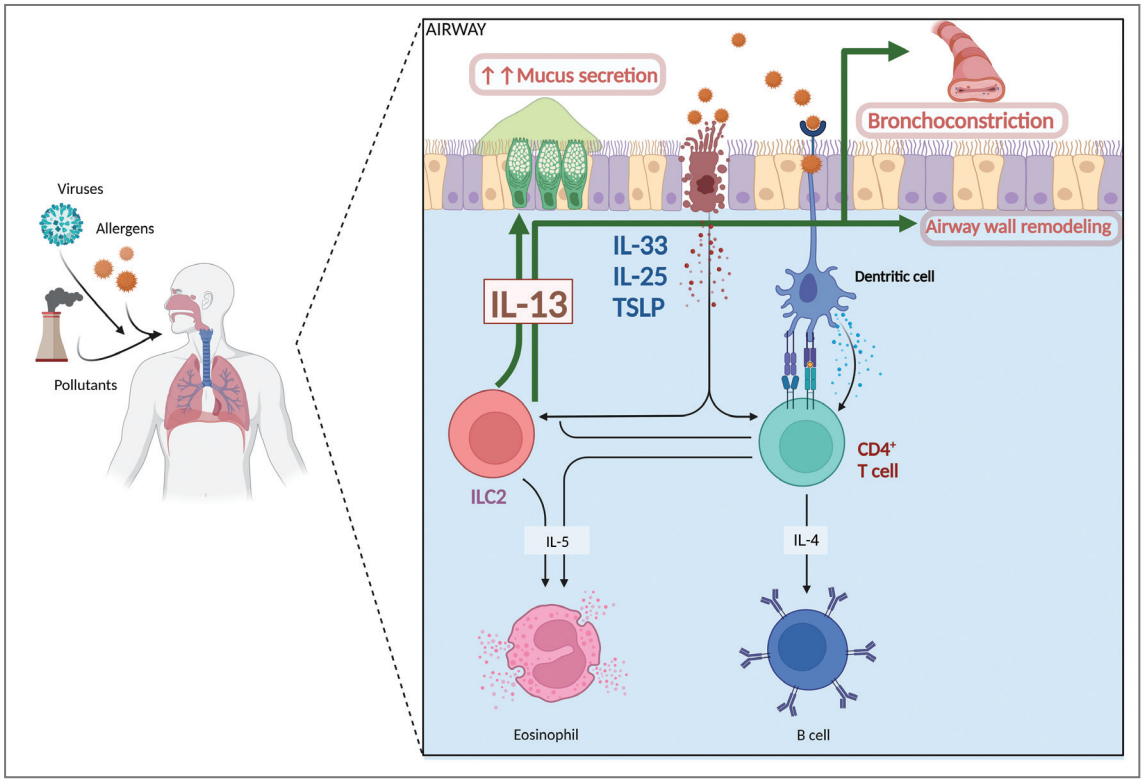

Fig. 1 IL-13 as a central effector cytokine of the T2-mediated inflammatory process, created in Biorender.com, **abbreviations:** IL-25: interleukin 25, IL-33: interleukin 33, TSLP: thymic stromal lymphopoietin, ILC2: natural lymphoid cell type 2

However, clinical cases of children with difficult-to-treat AB after treatment with systemic corticosteroids showed a more significant correlation with decreased FENO values than the number of eosinophil histological samples from the bronchial mucosa (31). Both FENO and eosinophils are closely associated with T2-type inflammation in patients with asthma. However, its production is regulated by different inflammatory cascades (IL-5: eosinophils versus IL-13: FENO)(32). Therefore, it is important to precisely define their specific roles in the etiopathogenesis of AB as independent, interrelated, and complementary biomarkers in the holistic concept of the disease.

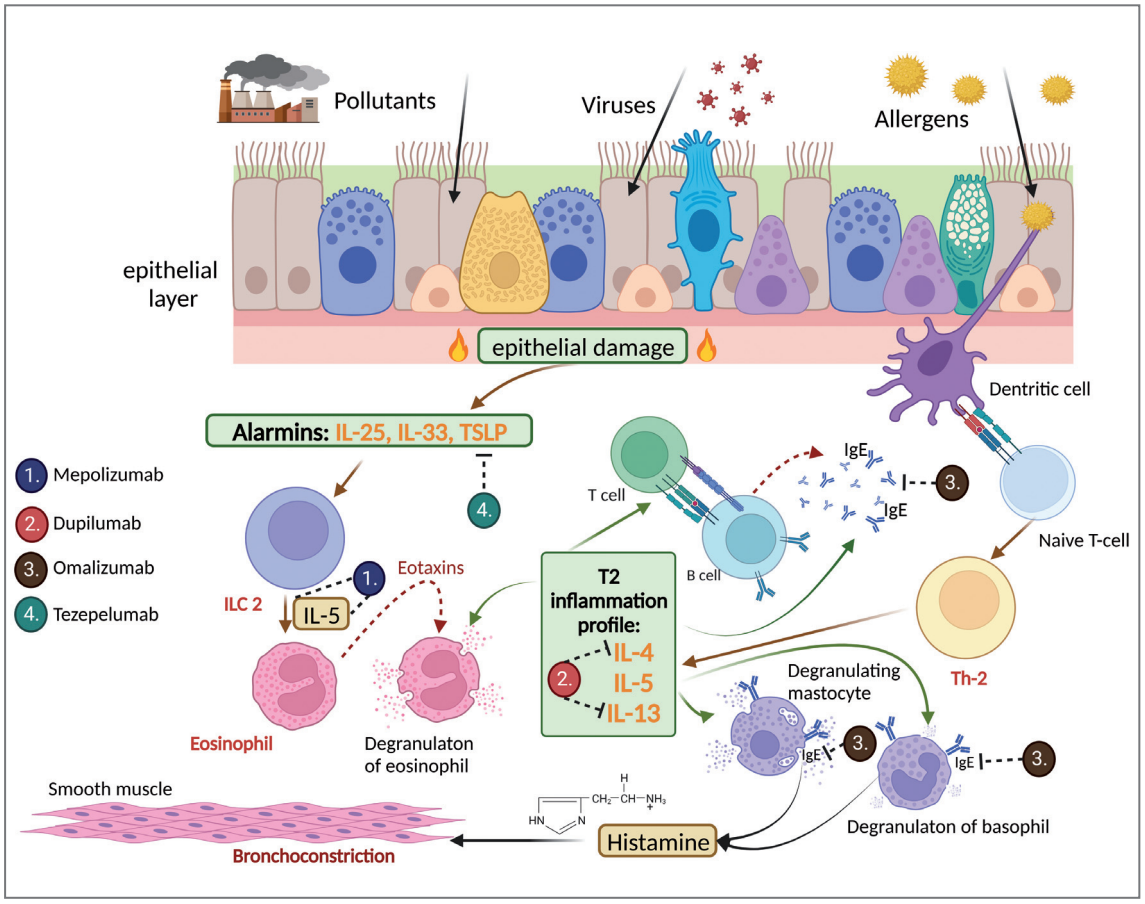

Fig. 2 Main pathways resulting in development of T2 type asthma and selected biologicals related to FENO, source: own elaboration, created in Biorender.com, **abbreviations:** IL-25: interleukin 25, IL-33: interleukin 33, TSLP: thymic stromal lymphopoietin, ILC2: natural lymphoid cell type 2, Th2: Th2 lymphocytes.

VARIABILITY OF FENO

FENO levels depend on various determinants and should be interpreted in the context of anamnestic data and clinical presentations. Factors with a possible impact on the measured values include atopy, height, age, sex, race, upper or lower respiratory tract infection, nitrate consumption in food, caffeine, alcohol, and active or passive smoking (Tab. 1) (33). Paradoxically, smoking and bronchoconstriction contribute to a decrease in these values.

Obesity is a growing global health problem and is negatively associated with asthma control. Dysregulation of metabolic and neuroendocrine processes and interactions in adipose tissue enhances inflammation in the body. Surprisingly, lower FENO values were confirmed despite significant sputum eosinophilia in obese patients (34). Exhaled NO undergoes diurnal changes simultaneously. Anderson et al. showed that the results of morning measurements were significantly higher than those of night measurements (35). While systemic and inhaled corticosteroids decrease FENO levels in children, antihistamines increase them (36). T2 inflammation-related diagnoses (atopic dermatitis, allergic rhinitis, and eosinophilic bronchitis) contribute to increased exhaled nitric oxide levels. Exercise is also an important factor. Clinical studies have demonstrated the effect of intense physical activity on higher FENO levels in less active children and adolescents (37). Finally, age should be considered when interpreting the results. The increase in age and height in specific age groups of the paediatric population determines the variation in FENO. A linear increase in the values was observed in girls aged 6–14 years. A similar linear relationship was also described for the opposite sex in the age group of 6–16 years. Both sexes reach a plateau of maximum concentration during adolescence, which persists into adulthood (38,39). Ethnicity and race also predict different basal levels of FENO in healthy and asthmatic children. In the US, Hispanic and African American children had higher mean FENO levels than Caucasian children (40).

Some studies have suggested the use of FENO measurements for the detection of cough-variant asthma and eosinophilic bronchitis in adult patients with chronic cough. Considering the many determinants of the variability of the FENO value, the importance of correctly assessing the results in correlation with selected anamnestic data has increased (41). The authors of this article investigated the possible association between FENO levels and cough reflex sensitivity in childhood asthma. There was no correlation between the elevated values of the parameters studied and the increase in the rate of cough reactivity in the asthmatic group or the control group of healthy probands (42).

The ability to investigate preschool-aged children is also clinically valuable. Based on the availability of devices using electrochemical analysis techniques, children above the age of five have a success rate of approximately 70% (43). In our clinical experience, most children over 8 years of age can handle this test.

Table 1 Various factors affecting FENO levels

FENO levels increase with:	FENO levels decrease with:
Allergic rhinitis	Early allergic reactions
Atopic dermatitis	Bronchoconstriction
White race	Corticosteroids (oral and/or inhaled)
Intake of a nitrate-rich diet	Passive and active smoking
Acute infection	Obesity
Morning (diurnal variation)	Reduced age in children
Male sex	
Caffeine	
Alcohol	

FENO AND SELECTION OF BIOLOGICS

Severe refractory asthma (subphenotypes of asthma that do not respond to current standard therapy) is a serious health problem. Pediatric asthma is often more treatable than adult asthma with adequate adherence to patient treatment. Severe asthma can also occur in children. Innovative biologics are available in cases where the disease control cannot be achieved despite the exhaustion of standard therapies. These drugs belong to a group of licenced monoclonal antibodies that block the T2 signalling pathway of inflammation. They act by disrupting associated T2 cytokines and IgE antibodies. Selective biomarkers can be used to identify suitable treatment responders. Omalizumab was the first approved monoclonal antibody for the treatment of asthma with a history of more than 20 years. This is an IgE-blocking IgG1 antibody. The mechanism of action involves interrupting the binding of IgE antibodies to coupled receptors on the cell surface. Omalizumab is indicated for add-on treatment of children aged six years of age. The role of FENO in selecting right patients and predicting a good therapeutic response remains unclear (44). A post-hoc analysis examining the effect of Omalizumab treatment on FENO levels yielded interesting conclusions. The study group of pediatric patients (>12 years) with higher FENO levels (>19.5 ppb) had a more significant reduction in exacerbations after 48 weeks of treatment (53% vs. 16%) compared to the group with lower FENO levels (45).

Eosinophils are therapeutic targets for the treatment of asthma. Since IL-5 is the major cytokine involved in eosinophil migration, maturation, and activation, the IgG1 monoclonal antibody Mepolizumab was specifically designed to target this cytokine. Treatment can also be initiated in children with severe eosinophilic asthma up to the age of 6 years. The outcomes of Mepolizumab treatment showed clinical evidence of efficacy in a real sample of paediatric patients with severe asthma when administered in routine care settings in Slovakia (46). The results of realized studies do not support the relevance of FENO as a biomarker of therapeutic success in patients treated with Mepolizumab (29,47). On the contrary, one study partially confounds the conclusions of previous clinical observations. A post-hoc analysis of the phase 2b DREAM trial data showed that adult patients treated with Mepolizumab with higher FENO (>25 ppb) and higher eosinophil (300 cells/μL) showed more significant reduction in exacerbations than the group with lower FENO (<25 ppb) and elevated eosinophils (62% vs. 34%) (48).

NO production is associated with the IL-13 signalling pathway. Dupilumab belongs to the family of biologics available for the treatment of AB. Dupilumab inhibits IL-4R signalling induced by both IL-4 and IL-13, and down-regulates T2 inflammation in a different allergic disorder (49). This dual mechanism inhibits the action of both pro-inflammatory cytokines in patients with T2-positive asthma. Dupilumab is indicated for patients aged 6 years and older with allergic and non-allergic eosinophilic asthma with FENO > 25 ppb, who depend on oral corticosteroids to control their symptoms, regardless of the blood eosinophil count (50). The role of FENO as a predictor of a good therapeutic response was confirmed in the Liberty Asthma Quest trial (NCT02414854). Probands aged 12 years and older with mean FENO values >25 ppb had a significantly reduced number of exacerbations (approximately 50%) compared with patients with FENO values <25 ppb (51). Furthermore, another study confirmed that a consistent decrease in baseline FENO values in adolescents and adults was associated with the chronic administration of anti-IL-4R . This suggests the potential of FENO as a pharmacodynamic biomarker for Dupilumab treatment (52).

Recently, Tezepelumab has been approved for the treatment of severe asthma. It targets one of the known epithelial cytokines, TSLP, which plays an important role in AB pathogenesis. The European Medicines Agency (EMA) has approved it for the treatment of adolescents aged ≥ 12 years and adults with severe asthma, regardless of phenotype, and without other indication limitations (positive biomarkers). Tezepelumab reduced eosinophil counts, FENO, and IgE in a clinical efficacy study of participating probands, suggesting its multi-suppressive action on individual inflammatory pathways. Similar to Dupilumab, patients

with higher FENO levels have significantly reduced disease flares (53). While innovative biologics are very effective in treating asthma, they do have some risks. These risks include an increased number of acquired infections, allergic and autoimmune reactions and in rare cases serious side effects (54).

Studies using biologics performed so far have focused on the usefulness of this biomarker in predicting therapeutic responses. Significantly less research has been devoted to assessing the significance of the dynamics of FENO values associated with treatment with these monoclonal antibodies. Indeed, the subject of future studies in this field may be its prognostic relevance to changes in biologics in the patient's therapeutic regime (55).

CONCLUSIONS

AB is a complex, multifactorial disease. Similar to other allergic disorders, the number of new cases diagnosed in children is expected to increase continuously in the future. In clinical practice, the diagnosis and care of pediatric patients with asthma is predominantly based on the evaluation of symptoms. However, this approach has some limitations. In many cases, this can lead to misdiagnosis. Therefore, methodologies that aim to assess the functional status of the airways, including bronchial hyper-responsiveness, have a unique place in the diagnostic algorithm of AB. However, these tests do not directly assess the severity of airway inflammation, which is the principal pathognomonic feature of AB.

FENO is a non-invasive, easy-to-implement, repetitive measurable, and economically rentable biomarker that accurately reflects the level of T2 inflammation in the bronchi. IL-13 production and activity in the airway epithelia affect the dynamic variability of FENO levels. This represents another additional tool to improve diagnosis, especially in paediatric patients with AB. FENO does not replace commonly used diagnostic options but complements them when appropriately indicated and interpreted. The application of this tool may be particularly beneficial in more severe and complicated cases of asthma. Knowledge of the relationship between decreasing FENO values in the context of the biological effects of inhaled and systemic corticosteroids suggests its role as a predictor of the possible therapeutic response and indirectly the assessment of adherence to treatment. Additionally, it may be helpful to stratify patients according to the future risk of the disease exacerbation and a decrease in spirometric

Table 2 Review article highlights

Highlights
➢ FENO as asthma biomarker reflects the level of T2 eosinophil inflammation in the bronchial tree.
➢ FENO is a non-invasive, easy-to-implement, repetitive measurable, and cost-effective biomarker.
➢ FENO can be measured by exhalation into an analyser in young children aged 5 years.
➢ Nitric oxide production in asthma inflamed airways is associated with the IL-13 signalling pathway.
➢ FENO levels depend on various determinants and should be interpreted in the context of the patient's history and clinical symptoms.
➢ FENO is helpful to recognise responders on corticosteroids and to follow the response and adherence to treatment.
➢ FENO can be useful biomarker to predict therapeutic response and efficacy in reducing the risk of future asthma exacerbations in candidates for biologic therapy with Omalizumab, Mepolizumab, Dupilumab and Tezepelumab.

parameters. Other potential use of FENO is to predict which children who have repeated episodes of wheezing are likely to be diagnosed with asthma in the future. New findings have revealed the importance of the FENO in the context of potential for positive therapeutic response and monitoring of treatment with selected biologics (Omalizumab, Mepolizumab, Dupilumab, Tezepelumab).

The implementation of more well-designed studies with sufficient evidence will help to better understand the importance and targeted implementation of FENO in cases of severe asthma treated with biologics.

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