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METHODS OF OBJECTIFYING COUGH

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Abstract

Cough is one of the most common symptoms encountered by clinicians. Attempts to measure cough date back to early 1950s and since then, significant progress has been made in understanding cough and many methods have been developed. Methods for cough measurement can be divided into subjective and objective methods and further according to the aspect of cough they assess. Subjective methods for cough assessment provide us with information about patient's personal experiences of cough and about psychosocial aspect of cough. Most widely used subjective methods include cough severity visual analogue scale, cough severity diaries, and various quality of life questionnaires. Objective methods for cough assessment focus mainly on cough frequency and on cough reflex sensitivity. Most widely used methods from this group include cough monitors and cough inhalation challenges. This review focuses on the most widely used cough measurement methods and points out their advantages and limitations for use in research and clinical practice. The ability to measure cough in clinical and research conditions could be used to determine treatment outcomes, to test new therapies, and to further study pathophysiology and physiology of cough.

Keywords: Cough, measurement, assessment, methods, tools

INTRODUCTION

Cough is defined as a three-phase expulsive motor act characterized by an inspiratory effort (first phase), followed by a forced expiratory effort against a closed glottis (second phase) and then by opening of the glottis and rapid expiratory air flow (third phase) (1, 2). Under physiological circumstances, cough is a complex protective reflex of respiratory organs triggered by mechanical or chemical stimulation of sensitive fibers in respiratory airways. The main function of cough reflex is disposal of various endo- and exogenic substances from respiratory airways and thus protection of lung and airways (3, 4). Despite coughs protective function, various conditions may lead to excessive and chronic cough that loses its protective function and negatively affects patient's health and quality of life (4, 5, 6). Cough is one of the most common reasons why patients seek medical attention, either a general practitioner or a respiratory specialist. Chronic cough is the most frequent reason why patients seek a respiratory specialist, while it is estimated that chronic cough affects approximately 10 percent of adult population worldwide (7, 8, 9, 10). Cough, especially chronic cough, is also associated with significant psychological and physiological morbidity (11).

METHODS FOR COUGH MEASUREMENT

During past two decades, significant advances in cough research were made including the development of tools for assessing and objectifying cough (7, 12, 13, 14, 15). Up to this day, there are various subjective and objective methods for cough assessment available. In order

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to achieve a complex assessment of cough (especially in research conditions), a combination of methods that focus on different aspects of cough shall be used (2). Out of all different aspects of cough, the most frequently investigated ones include: cough count and frequency, cough severity, impact of cough on health and quality of life, and cough reflex sensitivity (7, 12, 15).

SUBJECTIVE METHODS FOR COUGH MEASUREMENT

There are various validated subjective methods for cough assessment available including cough diaries, questionnaires, cough scores, or visual analogue scale (Tab. 1) (7, 15). All these methods assess cough by asking the patient about his/her perception of various aspects of cough including cough severity, cough frequency, impact of cough on his/her daily life (7). These subjective methods enable us to get information about the patient's personal experiences of cough and about psychosocial aspect of cough which are often overlooked and unrecognized (12). These methods are fairly simple and practical, especially in a longitudinal comparison, however they are not so reliable for a horizontal comparison and are susceptible to individual factors (9). Subjective methods for cough assessment can be further divided into two main groups according to which aspect of cough they focus on. The first group of methods focuses mainly on cough severity and the second group focuses on cough-related quality of life (QoL), which addresses the impact of cough on general health (12).

Cough severity assessment

Out of the subjective methods that focus on cough severity, the visual analogue scale (VAS) is the most frequently used tool for assessing cough severity both in clinical and research settings. The cough VAS consists of 100 mm linear scale marked from zero (which stands for no cough) to 100 mm (which stands for the worst possible cough), where patient can indicate the severity of cough. Higher the number on the scale means more severe cough (2, 7, 12, 15). This subjective method is very simple, brief, easily repeatable, responsive, and can be easily used in any clinical setting, especially for assessing chronic cough (2, 7, 12, 15, 16). However, this method did not undergo such rigorous validation as another methods like quality of life questionnaires (16). When compared to other subjective methods, there is only a moderate correlation between VAS and quality of life questionnaires. Furthermore, VAS does not relate well to cough reflex sensitivity and other objective methods (2).

Cough severity diary

Another subjective method for cough severity assessment is a cough severity diary (CSD). CSD is a simple daily seven-item diary developed to evaluate cough severity using feedback from patients. This tool correlates weakly with other objective methods assessing cough frequency, however, CSD outcomes strongly correlate with VAS scores. Furthermore, self-completed diaries for children correlate better with other objective tools and have a good responsibility. To fully assess usefulness, reproducibility, and responsiveness of CSD, further evaluation and exploration of this tool is needed (2, 12, 15, 17).

Quality of life questionnaires

The second main group of subjective methods for assessing cough comprises of health-related quality of life (HRQoL) questionnaires that focus on the impact of cough on patient's daily life, his/her physical and psychical health and the quality of life (7, 12, 15, 16). These tools enable the patient's perspective being brought into account (2). When compared to VAS, they capture a wider impact of cough on the patient (12, 15). There are many disease specific (cough specific HRQoL) and also many generic questionnaires available, however the usage of cough-specific tools is recommended in cases where cough is an important part of the symptomatology (2,7,15). It is because cough related questionnaires are simpler and

more responsive than generic questionnaires (2,12,15). Out of the cough-specific HRQoL questionnaires, the Leicester cough questionnaire (LCQ) and the cough-specific quality of life questionnaire (CQLQ) are most widely used either in clinical practice and research to assess longitudinal changes in patients with chronic cough and identify the specific health domains affected (2,12,15).

LCQ is a 19-item questionnaire addressing physical, psychical, and social domains, that has been used since 2001 (12,18). LCQ is a well validated, easy to use and score, and responsible tool that provides consistent repeatable results. However, when compared to VAS, there is only a moderate correlation between these tools. Furthermore, when compared to CQLQ, there is a weak to moderate correlation between the two tools because both tools assess a different aspect of impact of cough. This leads to their possible use as complementary tools for assessing cough (2, 12, 15, 18).

CQLQ is a 28-item questionnaire addressing 6 domains including functional abilities, physical complaints, extreme physical complaints, personal safety fears, psychosocial issues, and emotional well-being (12). CQLQ is a responsive, validated tool in acute and chronic cough with a high internal consistency, reliability, and repeatability used in clinical studies (including clinical trials evaluating antitussive therapies) that is also applicable in clinical practice (2, 12, 15, 16, 19). When compared to VAS, there is also a weak to moderate correlation between CQLQ and VAS. As a result of this, the European Respiratory Society (ERS) guidelines on cough measurement recommend the usage of cough VAS and quality-of-life questionnaires in combination to assess severity of cough and effect of cough on health in patients with chronic cough in complex manner (2).

Table 1 The most widely used subjective methods for cough assessment (modified by 7, 15)

Subjective methods for cough measurement	
1. Methods assessing cough severity	Visual analogue scale (VAS)
	Cough severity diary (CSD)
2. Quality of life questionnaires	Leicester cough questionnaire (LCQ)
	Cough-specific quality of life questionnaire (CQLQ)

OBJECTIVE METHODS FOR COUGH ASSESSMENT-COUGH MONITORING

There are variety of methods available for objective cough assessment that focus mainly on two aspects of cough including cough reflex sensitivity (assessed by cough challenge tests) and cough frequency (assessed by cough monitors) (Tab. 2) (9, 12). The first attempts by researchers to objectively measure cough (to quantify the amount of coughing per certain time period) date back to 1950s (2, 20). Up to this day, several ambulatory and non-ambulatory methods of cough monitoring have been developed and evaluated. The early methods for cough assessment in non-ambulatory conditions relied on tape recorders with microphones or on observers that count cough as they occurred in subjects. Assessing cough frequency by a manual counting of coughs from sound or video recordings still seems to be the most reliable method of objective cough assessment, however this method is very time consuming, laborious, and greatly limits the size and scope of studies (2, 12).

Thanks to the progress in hardware (battery life and recording capacity) and software development, various semi-automated or automated monitors that count cough over a certain

period of time (usually 24 hours) were developed. Leicester Cough Monitor (LCM) and VitaloJak are the most frequently used cough monitors in clinical research. These devices record sound for a certain time period (24 hour or longer) and after that, in VitaloJak the data are digitally processed and compressed to a much shorter recording which is then manually refined by a human operator. LCM is largely automated and only minor refinement by an operator is required (2, 7, 12, 15, 16, 21). The above-mentioned monitors were used in several clinical studies and proved to be useful, sensitive, and repeatable, with a potential to be used in clinical practice, for example to quantify the response to therapy. However, none of these monitors is standardized, clinically acceptable, or commercially available and thus is not deemed to be the gold standard for objective cough measurement in research nor in clinical conditions (2, 7, 12, 15, 16, 21).

Cough reflex sensitivity

Cough reflex sensitivity can be described as either as a reaction intensity of the cough reflex to different stimuli or a reactivity of afferent nerve cell endings in respiratory airways (7, 9). An abnormally high response of cough reflex towards various mechanical, thermal, or chemical stimuli or activation of cough reflex by lower levels of stimuli can be seen in many adults or children with various respiratory disorders, for example bronchial asthma. The exact reason of heightened cough reflex is unknown, there are many possible pathomechanisms involved including central and peripheral sensitization of cough reflex (4, 7, 9, 14, 22).

The cough reflex sensitivity measurement by inhalation cough challenges has proved to be an important component of many research studies, especially to determine the effect of various antitussive agents of cough reflex sensitivity (23). Various methods for assessing cough reflex sensitivity have been developed and tested, however only few of them are standardized. These methods induce cough either by a chemical or mechanical stimulation of airway afferent nerve endings. According to how cough is induced, the methods for measuring cough reflex sensitivity can be divided into two main groups: cough inhalation challenges and mechanical cough challenges, while the former group is most widely used. Stimulus intensity comparison or comparison of response to irritants are used to assess the levels of cough reflex sensitivity (7,9).

All inhalation challenges for cough reflex sensitivity measurement are in principle very similar to bronchial responsiveness assessment tests. Cough in subjects is induced by an inhalation of nebulised tussive agents (15). During past decades, many tussive agents have been used to induce cough reflex mainly by C-fiber activation. There are two main groups of inhalation challenges according to how the tussive agents are delivered during testing (single dose challenges and dose-response challenges) (21). The first group of inhalation challenges – single dose challenge-is based on inhalation of a single dose of tussive agent. These types of inhalation challenges are especially beneficial in epidemiological surveys, mainly because of their simplicity, relatively short duration, and no risk of significant adverse reactions. The second group of inhalation challenges – dose response challenges – can be further divided into two types according to the length of inhalation of a tussive agent: 1. single vital capacity breaths of incremental concentrations of tussive agents, 2. tidal breath inhalations of incremental concentrations of tussive agents during a fixed time period (9). The former type provides more accurate and repeatable results and thus is recommended to be used (24).

Cough challenge endpoints are usually expressed as C2 or C5, which stands for a tussive agent concentration that caused two (C2) or five (C5) coughs (23). Cough inhalation challenges proved to be very useful tool in both human and animal research studies providing good reproducibility and responsivity. Furthermore, cough inhalation challenges are valuable tool in measuring the ability of various antitussive agents to suppress cough (12). However, the inability of these cough challenges to discriminate patients with cough from healthy subjects is a major factor limiting their use in clinical practice as a diagnostic tool (15,16). Other limiting factors include inability to measure cough severity, raw material availability,

complex preparation process of tussive agents, requirement of nebulizer, and device-related maintenance (14,16). In order to ensure optimal results, all subjects also have to be properly instructed before the cough challenge (23).

Tussive agents used in cough measurement sensitivity

Tussive agents used to induce cough can be divided into non-acid and acid agents. Out of the non-acid agents, capsaicin is the most frequently used and recommended agent. Out of the acid agents, citric acid is the most commonly used agent.

Capsaicin

Capsaicin has been used as a tussive agent for several decades, either in human and animal research studies to assess cough sensitivity (5, 7, 9, 14, 15, 16). Capsaicin, a pungent substance commonly present in chili peppers, induces cough by transient receptor potential vanilloid-1 (TRPV1) activation (5,9). The usage of capsaicin as a tussive agent dates back to 1984 and during past three decades became a method of choice (9, 23, 25). There are several reasons why capsaicin is considered a tussive agent of choice, mainly due to its ability to induce cough in a dose-dependent manner, its reproducibility and safety. Furthermore, there are no serious adverse effects of capsaicin inhalation reported up to this day, most common adverse effect, transient throat irritation, was only present in a minority of subjects. Lastly, the usage of capsaicin as a tussive agent in patients with bronchial asthma is reported to be safe, tolerable, and without any clinically significant bronchoconstriction (23, 24, 26, 27). When compared to citric acid challenge, capsaicin inhalation lacks choking sensation and pharyngeal discomfort, also no significant tachyphylaxis has been reported (28). Inability to distinguish healthy subjects from patients with cough, inability to measure severity of cough and laborious process related to dilution preparation and device maintenance seem to be the most significant limitations of this method (14, 16).

Citric acid

Citric acid is the oldest and one of the most frequently used acid agents for cough induction that has been used since 1950s. The mechanism of cough induction by citric acid is not entirely described, however C-fiber, A-delta fiber, and TRPV1 receptor activation are the most probable mechanisms of cough induction. Citric acid inhalation challenge is safe, well tolerated, and feasible method of cough reflex sensitivity assessment (2,9,23,28). However, the presence of pharyngeal discomfort and choking sensation is more frequent when compared to capsaicin cough challenge. Furthermore, when the challenge is repeated over short 10-minute intervals, tachyphylaxis develops (15). The lack of guidelines and technical standards are other limitations of this method (28).

Table 2 The most widely used objective methods for cough assessment (modified by 7,15)

Objective methods for cough measurement		
1. Methods assessing cough frequency	A. Manual counting of coughs from sound or video recordings	
	B. Cough monitors	Leicester cough monitor (LCM)
		VitaloJak
2. Methods assessing cough reflex sensitivity	A. cough inhalation challenges	Acid tussive agents (citric acid)
		Non-acid tussive agents (capsaicin)
	B. mechanical cough challenges	

CONCLUSION

This review was focused on the evaluation of the most frequently used methods for cough assessment, where we pointed out their advantages and limitations. Past two decades brought us a significant development of various methods for cough measurement, which are already well established and standardized for use in cough research and clinical trials. Subjective methods of cough assessment are relatively cheap, simple, and validated, however they are still not widely used in clinical practice. Assessing cough frequency and cough reflex sensitivity measurement are also frequently used, especially in research and in clinical trials providing reliable results. However, there are many factors limiting the use of these objective methods in clinical practice or in larger scale research. COUGH-1 and COUGH-2 (29), the first- ever phase 3 trials of a novel treatment specifically for refractory chronic cough and unexplained chronic cough. Participants were required to have visual analogue scale, electronic cough severity diary, Leicester cough questionnaire was completed, and cough frequency was measured for 24 h. Future development may potentially bring us an improvement of already existing methods, which will make them suitable for use in research and clinical conditions and thus help us understand and treat cough better. The current use of cough objectification is primarily in clinical studies aimed at the treatment of chronic cough.

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IS NASAL NITRIC OXIDE MEASUREMENT AN USEFUL DIAGNOSTIC TOOL IN RESPIRATORY DISEASES?

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Abstract

Nasal nitric oxide (nNO), discovered in exhaled air in 1991, is produced in the nose and paranasal sinuses. This small gaseous molecule plays various roles in the organism, e.g. the first line defense through its antiviral and antimicrobial activity, stimulation of ciliary motility, regulation of pulmonary function. The measurement of nNO has become a diagnostic tool in various diagnoses, such as primary ciliary dyskinesia, allergic rhinitis, chronic rhinosinusitis, and others. In this article, we discuss the potential benefit of nNO measurement in diagnosis and monitoring of various respiratory diseases.

Key words: Nasal NO (nNO), Primary Ciliary Dyskinesia (PCD), Allergic Rhinitis (AR), Chronic Rhinosinusitis, Chronic Cough, Obstructive Sleep Apnea, Adenoid Hypertrophy

INTRODUCTION

The small gaseous molecule of nitric oxide (NO) is synthesized in the cells of the nervous system, cardiovascular system, and the upper and lower airways (1). NO plays multiple roles in many processes in the body including smooth muscle relaxation, vasoregulation, hemostasis, neurotransmission, immune defense, and respiration (2, 3). In 1991, NO was discovered in exhaled air (4). In the respiratory system, NO is synthesized from L arginine by NO synthase (NOS) which has three isoforms: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (3). The level of exhaled NO is usually increased and regulated by iNOS enzyme, induced by proinflammatory cytokines and/or bacterial products in almost every epithelial cell, while the other two isoforms are constitutively expressed (5, 6). In the airways, NO concentrations in the upper respiratory tract are higher than its levels in the lower respiratory tract (7).

The main production site of exhaled NO is the nose and sinuses (8). Nasal NO has important local and distal effects in keeping the eubiosis in the sinuses, contributing to local host defense, stimulating ciliary motility, and as an aerocrine mediator regulating pulmonary function by improving oxygen uptake and reducing pulmonary vascular resistance (9, 10). Nasal treatments such as polypectomy, sinus surgery, removal of hypertrophic adenoids and tonsils, and treatment of allergic rhinitis may alter NO output and, therefore, the microbial colonization of the upper airways. Nasal surgery aimed at relieving nasal obstruction

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may do the same but would also be expected to improve pulmonary function in patients with asthma and upper airway obstruction (2). Most studies indicated that the main production of nNO was in paranasal sinuses (5). However, NO is also produced in the nose at the apical tip of the ciliated respiratory mucosa. A study of Oh et al. localized nitric oxide production in the pericytes and osteocytes of nasal turbinates (11). Nasal NO concentrations are influenced by age, physical exercise, smoking, and certain drugs (7). Concentrations are increased in asthma, allergic rhinitis, and viral respiratory infections, but reduced in sinusitis, cystic fibrosis, primary ciliary dysfunction, chronic cough, and after exposure to tobacco and alcohol (2). At the respiratory level there are three different measurement options of NO: nNO, FeNO (exhaled fraction of nitric oxide), and CaNO (alveolar nitric oxide) (12). Studies have shown that CaNO is a potentially useful measurement for investigating the role of peripheral airway inflammation in asthma (13). The contributions of the bronchi (bronchial NO flux) and the alveoli (alveolar NO concentration) to FeNO were derived from regression analysis, with NO output as the dependent and exhalation flow rate as the independent factor (14).

The measurement of nNO is easy and non-invasive. There are two different ways of measuring the fractional concentration of nasal NO. If the measurement is obtained by nasal exhalation, it is called nasal FeNO. If the measurement is obtained by transnasal flow in series, it is called nNO (recommended by American Thoracic Society – ATS) (15). The air is analyzed by the NO analyzer using a chemiluminescence method, based on a reaction of NO with O3 resulting in the emission of light (7). Exhaled air from the lower airways has a much lower concentration of NO than the nose; thus, maneuvers must be instituted to close the soft palate to limit contamination of nasal air by air from the lower airways (16). The measurements should be performed when respiratory status is stable and then confirmed on a separate day (17). The side-related differences may be a consequence of e.g. impaired communication of the nasal cavity with sinuses at one side due to mucosal swelling or some other types of obstruction in osteomeatal complex. The role of the nasal cycle is important because it can influence the detection process (18).

nNO measurement is a highly feasible test in cooperative patients, generally >5 yr old. Unfortunately, up to this day, no single standardized procedure for nNO estimation has been defined (19).

Primary ciliary dyskinesia

Primary ciliary dyskinesia (PCD) is an autosomal recessive disease resulting in impaired mucociliary clearance associated with respiratory distress in term neonates, chronic otosino-pulmonary disease, male infertility, and organ laterality defects in ~50% of cases (20). This syndrome was initially recognized based on the triad of chronic sinusitis, bronchiectasis, and situs inversus (Kartagener syndrome) and Afzelius later recognized that these patients had “immotile” cilia and defective ciliary ultrastructure (21).

nNO is markedly reduced in this condition, with a diagnostic potential, being highly feasible, painless, noninvasive, rapid, and relatively inexpensive (22). nNO levels in patients with PCD are quite low (<77 nl/min), relative to normal values (range 125 to 867 nl/min; mean, 287 nl/min) (19). In the metaanalysis of 1,344 patients comprising 514 with PCD and 830 without PCD, diagnostic nNO cutoff values ranged from 16.8 to 100 nl/min, with a median cutoff at 76.9 nl/min (22).

It is critical to recognize that low nNO levels are also seen in some patients with cystic fibrosis (CF), even though nNO level in CF is not as low as in primary ciliary dyskinesia. Therefore, CF needs to be ruled out by sweat testing or CFTR genetic studies if nNO is low (21). However, in some cases, normal nNO levels do not rule out PCD, and patients with highly compatible PCD clinical phenotypes but normal nNO levels should be subjected to further testing (22).

Allergic rhinitis

Allergic rhinitis (AR), the most common allergic disease in both adult and pediatric population, is characterized by sneezing, itching, nasal congestion, and rhinorrhea following an exposure of allergens (5). Due to the chronic and recurrent inflammatory process in the sinonasal complex, nNO level is supposed to be significantly higher. Unfortunately, the detection of nNO as a marker in allergic rhinitis is still not validated for routine clinical use (18). Several studies have been performed to define the cut off value of nNO in allergic rhinitis patients. In the meta analysis of Wang et al., including 10 original studies with 561 AR patients, 327 healthy controls, and 123 NAR patients, the authors found that nNO in AR was significantly higher than in the healthy controls or NAR patients (5). Similar results were published by Chen et al, examined 55 cases of preschool children with AR. The levels of nNO were significantly higher in children with allergic rhinitis compared to the control group (23). The similar cut – off value (169.4 and 161.4 nl/min, respectively) was reported by two studies of Nesic et al. and Wen et al. (24, 25). The study of Antosova et al. confirmed the increase of nNO in allergic subjects during the pollen season and also out of it (18). However, some studies did not report a statistically significant difference of nNO levels in AR patients compared with the healthy controls. The probable cause is the swelling of nasal mucosa, leading to occluded sinus ostia and the blockage of NO distribution to the nasal cavity. For the same reason, nNO could not detect AR patients concomitant with nasal polyps, sinusitis, or marked ostial obstruction (5). The values of nNO in allergic rhinitis patients are also affected by medication. In the study of Antosova et al., the antiinflammatory treatment by the combination of antihistamines and nasal corticosteroids led to the significant decrease of nNO and this effect was more significant for women (18). Intranasal steroids therapy significantly reduces NO production by blocking the transcription of the iNO-synthase gene (26).

However, other studies failed to show the effect of nasal corticosteroid treatment on nNO levels (27, 28). This conflicting result is likely caused by diffusion impairment, extensive mucus production, mucosal swelling, impaired communication with sinuses, or occlusion of the nostril and application of decongestants (29). There are also studies documenting the elevation of nNO levels after corticosteroid treatment in participants with chronic inflammation and nasal polyps (18), which is probably caused by the reduction of swelling, opening ostiomeatal complex, and the release of nNO from maxillary ostia (30).

Liu et al. compared nNO values in patients with allergic rhinitis (AR) and nonallergic rhinitis (NAR) and the impact of sinus inflammation. Patients with allergic rhinitis without sinus inflammation showed the highest nNO levels and patients with non-allergic rhinitis with sinus inflammation had the lowest nNO levels (31).

As we see, the variable obstruction of ostiomeatal ostia and the medication have a significant impact on the nNO levels.

Chronic rhinosinusitis

Chronic rhinosinusitis is an inflammatory disease of the nose and paranasal sinuses defined by the presence of at least two out of four cardinal symptoms (i.e., facial pain/pressure, hyposmia/anosmia, nasal drainage, and nasal obstruction) for at least 12 consecutive weeks, in addition to objective evidence (32).

Despite the absence of reference values, lower nNO levels were observed in case of the chronic rhinosinusitis with nasal polyps (CRSwNP) and also without them (CRSsNP). CRSwNP patients exhibit significantly lower nNO values as compared to both CRSsNP and healthy subjects (33). In patients with CRSsNP were documented decreased nNP values with respect to the healthy subjects (26). It is uncertain whether the low NO levels detected in chronic rhinosinusitis result from a reduced maxillary NO production or are rather mainly due to an obstruction of sinus ostia (34). However, restoring patency of sinus ostia by endoscopic surgery has shown to be associated with a rapid increase in nNO levels (35). Hence, the low nNO levels can be explained through the nasal obstruction resulting from mucosal swelling (36). Another reason is the damage of the NO-producing sinus mucosa by

Table 1 nNO values according to diagnoses compared with healthy controls

Diagnosis	nNO values (ppb)	Healthy controls	Study
PCD	64±36.6	759±145.8	Wodehouse et al, 2003
Allergic rhinitis	939 ± 335	–	Liu et al., 2020
UACS with sinusitis	190.1 ± 114.8	334.9 ± 88.2	Kim et al., 2011
UACS without sinusitis	345.7 ± 114.6	334.9 ± 88.2	Kim et al., 2011
OSAS before sleep	487.03 ± 115.83	413.37 ± 73.10	Zhang et al., 2019
OSAS after sleep	550.07 ± 130.24	460.43 ± 109.7	Zhang et al., 2019
Adenoid hypertrophy gr. 1	814 (median)	1050 (median)	Chladkova et al., 2015
Adenoid hypertrophy gr. 2	728 (median)	1050 (median)	Chladkova et al., 2015
Adenoid hypertrophy gr. 3	539 (median)	1050 (median)	Chladkova et al., 2015

an increased synthesis of cytotoxic agents in chronic inflammation (37). The reduced expression of the inducible isoform of NO synthase (iNO-synthase) caused by some inflammatory cytokines (IL-4, IL-6, and TGF-β) has been found in the sinus mucosa of chronic rhinosinusitis patients (37). An increased arginase activity was confirmed in patients with chronic rhinosinusitis, that may decrease NO levels by means of reduced availability of L-arginine, the main NO precursor (38). Adult patients with CRSwNP have high levels of iNOS in the nasal epithelium due to inflammation of nasal and paranasal cavities, they exhibit reduced levels of nNO in comparison with subjects affected by uncomplicated allergic rhinitis (39). Lower nNO levels in patients with paranasal sinus inflammatory diseases are caused by mechanical obstruction of the draining ostia and by the negative pressure within the sinuses, resulting in a reduced transit of NO from sinuses to the nasal lumen, despite the increased NO synthesis due to inflammation.

Chronic cough

Chronic cough is defined as a cough that lasts for eight weeks or longer (40). Most common causes of chronic cough are asthma, gastroesophageal reflux disease, and upper airway cough syndrome (previously known as postnasal drip syndrome), collectively known as 'diagnostic triad of chronic cough' (40).

Manisalco et al. studied the FeNO and nNO in patients with chronic cough, classified to four cathegories (cough variant asthma (CVA), non-asthmatic eosinophillic bronchitis (NAEB), gastrooesophageal reflux disease (GERD), and upper airway cough syndrome (UACS). FeNO value was significantly higher in CVA and NAEB compared to GERD and UACS. However, no differences were found in nNO levels among the four groups and also in comparison with control groups (41). The explanation can be a continuous NO gas exchange between nasal cavity and the sinuses. The high NO levels in paranasal sinuses could easily blunt slight alteration of NO (42).

Similarly, in the study of Kim et al., the levels of nNO were not elevated in patients with UACS compared with other causes. UACS is not a single disease entity, this term refers to a variety of diseases, such as allergic rhinitis, nonallergic rhinitis, nonallergic rhinitis with eosinophilia, and bacterial sinusitis (43).

Among UACS patients, the concentrations of nNO in patients with sinusitis were significantly lower than in those without sinusitis. Furthermore, the levels of nNO in sinusitis

were much lower than those in non-UACS; thus, the measurement of nNO discriminated sinusitis from non-sinusitis causes in patients with prolonged cough (43).

Obstructive sleep apnea

The most important characteristic of obstructive sleep apnea (OSA) is repeated pharyngeal collapses during sleep. Local inflammation at nose, pharynx, and larynx aggravates upper airway narrowing and increases the risk of OSA (9). The most important mechanisms of upper airway inflammation in OSA is hypoxia-reflexogen-induced inflammatory cytokine release (44). The study of Zhang et al. observed the positive correlation between nNO and the percentage of neutrophils, IL-6, IL-8 in nasal lavage, which indicates that nNO is a marker of the severity of OSA (9).

According to the same study, both FeNO and nNO levels were significantly higher in OSA patients than in the controls (9). Moreover, FeNO and nNO are increased after sleep in OSA patients, while nNO is also slightly increased after sleep in the healthy group. The levels of nNO before and after sleep were closely related with sleep apnea severity (9). Compared to the healthy controls, nNO is also increased in children with sleep-disordered breathing, but it is not correlated with disease severity. This is probably due to the local mechanical processes and snoring (45).

Smoking and OSA have opposite effects on FeNO and nNO level, so the concentration of FeNO and nNO in smoking OSA were close to the normal range (9).

Adenoid hypertrophy

In adenoid hypertrophy (AH), clinical utility of nasal nitric oxide is still critically questioned and remains to be established. Chladkova et al. studied the adenoid hypertrophy as a factor that influences nNO values in children with PCD. The results show that nNO of patients with AH is less than in the healthy controls and decrease with its grade (46). This result was confirmed by Rybnikar et al. in the study of 48 non allergic patients. According to this study, nNO and FeNO were reduced in nonallergic children with obstructive adenoids and the median of nNO decreased with the increasing grade of adenoids. Following adenoidectomy, nNO level increased (47). As we see, adenoid hypertrophy can potentially cause a false positive result of the test for PCD (47). Bugova et al. studied nNO in 60 children with adenoid hypertrophy. The value of nNO in younger children was significantly lower. Children with confirmed bacterial nasopharyngeal colonization had significantly higher measured values of nNO (48).

CONCLUSION

The detection of nasal NO is supposed to be a beneficial diagnostic tool in respiratory diseases, being painless, noninvasive and rapid. However, nNO concentration is influenced by many factors mentioned above. Besides the primary ciliary dyskinesia, the detection of nNO is still not validated for routine clinical use. Further studies are needed to assemble the guidelines, define the cut-off values and specify the conditions for nNO measurement in clinical practice.

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SEARCH FOR MOLECULAR BIOMARKERS OF PARKINSON'S DISEASE. NEW TISSUES AND METHODS

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Abstract

Parkinson's disease (PD) is a chronic neurodegenerative disorder that is clinically manifested by motor and non-motor symptoms. At the early stage of the disease, it can be misdiagnosed with some neurologic disorders due to overlapping or similar clinical features. In addition, the pathogenesis of this disease is initiated several years prior to the appearance of classical motor symptoms. This latent phase of neurodegeneration in PD characterised at cellular level by preservation of significant fraction of dopaminergic neurones is of particular interest with respect to the development of disease-modifying or neuroprotective therapies which would require intervention at the earliest stages of disease with an aim to slow down or reverse the disease progression. Therefore, huge effort was performed in order to find and validate a biomarker that would reliably differentiate PD from other neurologic diseases as well as a biomarker that would reveal preclinical/prodromal stage of PD. This short review summarises a recent progress in validation of molecular biomarkers of PD, distinct from genetic markers of PD, with some focus on new analysed tissues and new methods.

Key words: Parkinson's disease, neurodegeneration, biomarker

INTRODUCTION

Parkinson's disease (PD) is the second most frequent chronic neurodegenerative disorder that is clinically manifested by motor and non-motor symptoms. At the pathological level, PD is characterised by the loss of dopaminergic neurons within the substantia nigra pars compacta (1) and the deposits of α-synuclein in a misfolded state that aggregates and forms intracellular inclusions within the cell bodies (Lewy bodies) and processes (Lewy neurites) of affected neurons (2). Other pathophysiologic features include mitochondrial dysfunction, impaired lysosomes or vesicle transport, impaired synaptic transport, and neuroinflammation (3). Finally, recent molecular genetic studies have revealed that genetic factors, in addition to aging and environmental factors, play an important role in the development of PD (4).

At the early stage of the disease, PD often presents similar clinical manifestations as multiple system atrophy (MSA) that is also categorized as α-synucleinopathy and often makes precise differentiation and diagnosis of these conditions difficult. PD can also be misdiagnosed with atypical conditions such as progressive supranuclear paralysis (PSP) due to overlapping clinical features. In addition, on the basis of converging results from clinical, neuropathological, and imaging research, it has been suggested that PD-specific pathology is initiated several years prior to the appearance of classical motor symptoms (5). This latent phase of neurodegeneration in PD characterised at cellular level by a preservation of significant fraction of dopaminergic neurones is of particular interest with respect to the development of disease-modifying or neuroprotective therapies which would require intervention at the earliest stages of the disease with an aim to slow down or reverse the disease

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progression. Therefore, there are two main challenges related to the molecular biomarkers of PD:

1. To find and validate a biomarker or set of biomarkers that would reliably differentiate PD from other neurologic diseases namely from atypical parkinsonian syndromes (APS), such as MSA, PSP, and corticobasal degeneration (CBD).
2. To identify and validate a biomarker or set of biomarkers that would reveal preclinical/prodromal stage of PD.

In addition, some effort is devoted to the identification of biomarkers that would monitor the efficiency of treatment and/or would be indicative for personalised treatment (6).

This short review is focused on the recent findings related to the validation of molecular biomarkers of PD with some focus on new methods of analysis or new analysed tissues. This review does not deal with biomarkers from a genetic point of view, which play a role in juvenile-onset and early-onset as well as late-onset adult PD caused by mutations in certain genes. Such mutations representing monogenically inherited PD are suitable biomarkers for an early detection of PD (4).

Alpha-synuclein

Alpha-synuclein is a small protein expressed at the nerve terminals where it is involved in the regulation of synaptic functions and neurotransmitter release (7). Its structure and function are extensively modulated by posttranslational modifications including phosphorylation as well as conformational changes that might result in the formation of protein aggregates and consequent death of neurones (8). Due to its central role in PD pathophysiology, α-synuclein represents the main protein biomarker related to PD (9). In terms of search for PD biomarkers, previous effort was focused on sensitivity and specificity of detection of either α-synuclein or α-synuclein phosphorylated at serine 129 in different tissues including gut, skin, *cerebrospinal fluid* (CSF), and blood. In addition to classical methods of α-synuclein detection (immunohistochemistry, immunofluorescence, and enzyme linked immunoassays), new methods such as real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA) were used recently (10, 11).

Recent meta-analysis showed a high degree of association between α-synuclein detected in biopsy samples from gastrointestinal tract (GIT) and PD (12). But it was also suggested that the measurement of GIT α-synuclein alone could result in the underdiagnosis of PD and combination GIT α-synuclein with other biochemical markers could increase specificity and sensitivity of examination (12). Another meta-analysis, comparing different α-synuclein antibodies as well as GIT and skin samples, has shown that antibodies against phosphorylated α-synuclein exhibit higher specificity than anti-native α-synuclein antibody (13). In addition, the same study revealed that the skin biopsy examination using antibodies against phosphorylated α-synuclein has the best diagnostic accuracy (13). With respect to the new methods, both RT-QuIC and PMCA assays of phosphorylated α-synuclein aggregation seeding activity in skin can differentiate synucleinopathies (PD, Lewy body dementia (LBD), and MSA) from non-synucleinopathies (Alzheimer disease, PSP, CBD, and non-neurodegenerative controls) with high sensitivity and specificity (14). Sensitivity and specificity of α-synuclein determination in skin biopsy as a potential diagnostic tool in PD as well as current methodological problems were summarised recently (15). Deposit patterns of either α-synuclein or phosphorylated α-synuclein in skin correlate well with clinical phenotypes in PD patients and can serve as a reference for the diagnosis and classification of PD (16).

Deposits of either α-synuclein or phosphorylated α-synuclein were found in CSF, however, a meta-analysis did not confirm a significant difference in total α-synuclein between patients with PD and other synucleinopathies or APS (17). The combinations of either total α-synuclein with NFL (18) or total α-synuclein with amyloid beta 1-42 peptide and NFL (19) could be promising approach for the differential diagnosis of PD and APSs.

Recent studies have revealed abnormal deposits of phosphorylated α-synuclein in both CSF (20) and dermal nerve fibres (21, 22) in persons with REM sleep behaviour disorder (RBD). Thus, phosphorylated α-synuclein seems to be a reliable candidate biomarker to screen

for prodromal PD during enrolment in trials of disease-modifying, α-synuclein-based therapies (10). Deposits of α-synuclein can also be analysed in biopsy samples of other peripheral tissues, such as submandibular or minor salivary glands and submucosal enteral tissue, however, these biopsies are more invasive than skin biopsy (23, 24, 25).

With respect to invasiveness of sample collection, blood represents the most suitable tissue. Since α-synuclein is also expressed in erythrocytes (26), conflicting data about the level of α-synuclein in blood of PD patients were obtained (6). Detection of exosomal α-synuclein or determination of α-synuclein oligomers as well as α-synuclein phosphorylated at serine 129 represents possibilities to increase sensitivity and specificity of blood α-synuclein as a biomarker for PD (8). Currently there is not a report focused on determination of blood α-synuclein seeding-capacity determined by either RT-QuIC or PMCA (10, 11). Saliva represent another body fluid that can be easily collected. Recent pilot study showed promising results indicating association between salivary α-synuclein seeding-capacity determined by RT-QuIC and PD (27).

Neurofilament light chain

Neurofilament light chain (NFL) is a structural protein that is expressed at high levels in axons, therefore NFL is a sensitive biomarker of axonal injury; however, its specificity is lower (28).

Recently performed meta-analysis suggested that the level of NFL in CSF could provide important information for the differential diagnosis of PD and APS, but NFL levels in CSF were found comparable between PD and control samples (19, 29).

Blood concentration of NFL is also increased in APS (30, 31, 32, 33) compared to PD. Determination of blood NFL has high potential to serve as biomarker with high accuracy levels to differentiate APS from PD, even in early stages of APS, when clinical symptoms are not yet conclusive (31, 32). Serum NFL concentrations correlate with age (30, 32, 33) in PD and controls, but not in APS (32). They seem to be higher in more advanced PD patients compared to controls, while controversial data were obtained for early disease stages (6). Heterogeneous data were obtained with respect to association of blood NFL concentrations with motor impairment in PD, revealing both positive (30, 34, 35) and negative results (36) while consistent positive association between baseline blood NFL levels and negative cognitive outcome have been reported (30, 36, 37).

Tyrosine hydroxylase

Tyrosine hydroxylase (TH) as a key regulatory enzyme involved in dopamine synthesis was considered as a good marker of degeneration of dopaminergic neurones. Despite this fact, the utilisation of TH as a biomarker of PD was investigated in a few studies several decades ago. Recent studies unexpectedly revealed that monocytes isolated from blood of PD patients express significantly higher levels of TH in comparison to age-matched healthy controls (38). It was suggested that the dopaminergic machinery on peripheral immune cells displays an association with human PD, with some implications in facilitating diagnosis. Higher expression of TH in monocytes was attributed to high levels of tumour necrosis factor α (TNFα) (39). Therefore, diagnostic accuracy of monocyte TH is not yet clear, since other diseases characterised with higher TNFα expression like multiple sclerosis might be associated with higher expression of TH in monocytes.

MicroRNAs

MicroRNAs (miRNAs) are endogenous small (18–25 nucleotides), single-stranded, non-coding RNAs that are playing a key role in post-transcriptional regulation of gene expression. Recently, different types of miRNA are considered to play a role in pathophysiology of PD (40), therefore significant effort was focused on identification and validation of miRNAs that can serve as biomarker of PD.

One of the first study revealed that miR-195 was up-regulated, and miR-185, miR-15b, miR-221 and miR-181a were down-regulated in plasma of PD patients thus this set of five

miRNAs can precisely distinguish PD patients from healthy individuals (41). Three plasma miRNAs (miR-671-5p, miR-19b-3p, and miR-24-3p) were found to be differently presented in MSA and PD having potential to become markers that could reflect the pathophysiology or symptoms of PD and MSA (42).

A group of three exosomal miRNAs (miR-21-3p, miR-22-3p, and miR-223-5p) discriminated PD from control while another group of exosomal miRNAs (miR-425-5p, miR-21-3p, and miR-199a-5p) discriminated PSP from PD with good diagnostic accuracy. A combination of the levels of six exosomal miRNAs (miR-21-3p, miR-199a-5p, miR-425-5p, miR-483-5p, miR-22-3p, and miR-29a-3p) discriminated PSP from PD with a relatively high sensitivity and specificity (43).

Marques and collaborators have identified two miRNAs in CSF (miR-24 and miR-205) that accurately discriminated PD from controls and four miRNAs (miR-19a, miR-19b, miR-24, and miR-34c) that differentiated MSA from controls (44).

Groups of three microRNAs in CSF discriminated PD (miR-7-5p, miR-331-5p, and miR-145-5p) and MSA (miR-7-5p, miR-34c-3p, and miR-let-7b-5p) from controls with good diagnostic accuracy. The combination that best distinguished MSA and PD consisted of two microRNAs (miR-9-3p and miR-106b-5p) while a single microRNA in the CSF (miR-106b-5p) exhibited the best discrimination between PD and PSP (45).

Since the panel of several miRNAs can be easily analysed via specific miRNA qPCR array, the determination of target miRNAs represents a promising diagnostic approach.

Mitochondrial DNA and proteins

Mitochondrial dysfunction is often implicated as an early event in pathophysiology of PD although the cause-consequence relationships are not fully clear (46). Mitochondrial dysfunction is often a result of endoplasmic reticulum (ER) stress (47) that is also suggested to be involved in the pathophysiology of PD. Unlike molecular determinants of mitochondrial dysfunction, alterations of some critical molecular components of ER stress pathways were explicitly detected in brains of PD patients (48, 49) previously and ER stress-regulated proteins were recently suggested as blood biomarkers to confirm the diagnosis of PD (50). Recent study has identified several proteins associated with mitochondrial functions that are significantly altered in brains of PD patients (46) but their use as biomarkers for PD will require additional experiments and validations.

Despite some critical view (51), the content of mtDNA in leukocytes could reflect the relative content of mitochondria in the affected peripheral tissue. For example, in the largest investigation of mtDNA copy number associated with PD, the reduced mtDNA copy number was documented exclusively in the substantia nigra pars compacta of PD patients but not in other brain regions. The same study has shown that the reduced mtDNA documented in brain was also reflected by the decreased mtDNA copy number in the peripheral blood cells (52). However, the decreased mtDNA in leukocytes was also documented in other diseases such as Alzheimer's disease (53), bipolar disorder (54), and schizophrenia (55). Finally, the decreased mtDNA copy number could be a useful biomarker of mitochondrial dysfunction associated with a number of different pathological conditions including PD (56). Although, the reduction in mtDNA copy number found in substantia nigra dopaminergic neurons of idiopathic PD patients was related to the dysfunction of mitochondrial respiratory chain (57), the recent pilot study has documented increased both maximal respiration and respiratory capacity in leukocytes of PD patients (58). Thus, the potential of PD biomarker based on mitochondria seems to be questioning.

CONCLUSION

In addition to the above discussed molecules, some other molecules were investigated as potential biomarkers of PD including inflammatory cytokines, amyloid peptide, and tau protein (6). With respect to the high throughput methods, OMICS approach involving

metabolomics (59) or proteomics (60) were investigated but their clinical use is hampered due to technologic and financial demands. Apart from molecular biomarkers, methods/protocols based on tissue imaging (e.g. fMRI, PET), allowing functional examination of brains of PD patients, are investigated in terms of differential diagnosis (61, 62).

Despite strong effort, a reliable biomarker that exhibit a high sensitivity and specificity for diagnostic and/or therapeutic purposes in PD does not currently exist. Such situation is probably a result of the high heterogeneity of PD caused by both phenotype and genotype or epigenetic factors. Further analyses of molecules in larger cohorts and in different samples are required for identification and validation of useful and reliable clinical biomarkers. In order to increase discrimination power, a combination of different biomarkers or a combination of molecular methods with imaging methods represents the possibility how to increase accuracy of PD diagnosis and management.

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VOLUMETRIC ABSORPTIVE MICROSAMPLING TECHNIQUE IN THE LC-MS DETERMINATION OF DIRECT ORAL ANTICOAGULANTS

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Abstract

Direct oral anticoagulants represent a significant group of drugs used in the prevention or treatment of venous thromboembolic events and stroke in patients with atrial fibrillation. Although routine therapy monitoring is not required, there is an increasing evidence that plasma levels may vary between individuals, suggesting the benefit of plasma levels measurement in some situations. Therapeutic drug monitoring is becoming more popular and accessible to the broader population. Introducing microsampling techniques for the quantitative collection of blood samples has arisen nowadays. The volumetric absorptive microsampling approach using a commercially available device such as a Mitra stick overcomes the hematocrit effect present in the dry blood spot technique. This review discusses the possible application of the volumetric absorptive microsampling approach in monitoring direct oral anticoagulant therapy efficacy.

Keywords: direct oral anticoagulants, microsampling techniques, therapeutic drug monitoring, volumetric absorptive microsampling

INTRODUCTION

Direct oral anticoagulants are currently widely used in many indications to prevent various thromboembolic events. Compared to vitamin K antagonists anticoagulants, routine therapeutic monitoring is not required; however, increasing evidence suggests the benefit of plasma levels measurement [1,2]. Moreover, laboratory monitoring might help improve patient and drug non-compliance [3]. Traditional blood sampling via venipuncture is an invasive and laborious process requiring trained personnel and subsequent processing of samples like centrifugation or freezing. Microsampling techniques appear as a promising alternative due to advantages such as quick sampling time, simplicity of sample transportation and storage, and they are less invasive [4]. This review is focused on an introduction to microsampling techniques and their possible application in the routine monitoring of direct oral anticoagulants using liquid chromatography coupled with mass spectrometry.

Microsampling techniques in therapeutic drug monitoring

Therapeutic drug monitoring (TDM) represents the clinical tool for optimizing individual dosage regimens for a specific patient. In general, TDM is defined as the measurement of clinical parameters which, together with an appropriate interpretation, might directly

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influence drug prescribing [5]. Through the combination of pharmacodynamics and pharmacokinetics knowledge, TDM enables the assessment of therapy efficacy and individualization of the treatment. TDM is based on the assumption of an exact relationship between dose and drug concentration in the biological matrix (e.g. blood plasma, whole blood, urine, etc.) as well as between concentration and clinical effects [6].

The conventional blood sampling technique is an invasive, tedious, and labour-intensive process. The venipuncture sampling technique is inconvenient and painful, especially for children and critically ill patients [7]. Moreover, trained medical personnel is required to take blood samples. Collected blood samples must be pre-treated as they are not analyzed immediately. This step usually involves centrifugation, aliquoting, freezing, and delivery of samples to a diagnostic laboratory. All mentioned factors increase the risk of errors, are time-consuming, and raise the analysis price [8,9]. Microsampling techniques are a minimally invasive alternative to venipuncture for collecting human body fluids specimens, especially blood samples [7]. Dried blood spot (DBS) sampling is a widely used approach in the research phase of drug development, preclinical toxicokinetic studies, and routine clinical and biochemical examinations. The DBS technique was first introduced to screen phenylketonuria, a metabolic disorder, in the neonatal population in 1963 [10,11]. The sample processing involves using a sample collection card and blood lancet. Briefly, after a finger-prick (or prick of the heel in infants) with the lancet, several drops of blood are absorbed onto the sample collection card, followed by drying, storage, shipping, and extraction steps before the analysis [12]. The shipping and storing conditions are simple and might be performed under ambient temperature and humidity in most applications [13]. Despite many advantages, several limitations arise. First, the vast majority of biochemical and analytical methods require plasma or serum samples; thus, the already existing methods and assays must be redesigned and revalidated specifically for DBS. Next, hematocrit of the blood affects viscosity and may introduce bias in the sampling step, impacting the extraction procedure and, thus, negatively influencing the assay. The spot area on the sampling card typically has a linear, inverse relationship to the blood hematocrit – blood with a high hematocrit level results in a smaller dried blood sample and vice versa [14,15].

During the last two decades, numerous alternatives to DBS have been developed mainly to diminish the hematocrit effect as well as to bring accurate microsampling devices in science and medicine [16]. The new dried blood sampling devices include volumetric absorptive microsampling (the device is called Mitra, produced by Neoteryx, USA), Capitainer qDBS (produced by Capitainer, Sweden), hemaPEN (produced by Trajan Scientific and Medical, Australia), HemaXis DB 10 (produced by DBS System SA, Switzerland), and HemaSpot HF (produced by Spot on Sciences, USA) [17]. In the next section of this review we focused on the volumetric absorptive microsampling technique which is rising in popularity nowadays in the fields of medicine and science.

Volumetric absorptive microsampling

Volumetric absorptive microsampling (VAMS) is a minimally invasive sampling technique for collecting peripheral blood. The device called Mitra was introduced in the market in 2014 by Neoteryx company (USA). The same year, Denniff and Spooner published a paper describing the VAMS approach using Mitra tips as an alternative microsampling method to DBS [18]. The Mitra tip consists of a hydrophilic polymer fixed to a plastic holder (Figure 1A), allowing the collection of the desired volume by capillary mechanism. Three volumes can be collected depending on the selected tip size – 10, 20, and 30 µL. The sample collection workflow consists of wicking the blood, which takes 3 – 5 seconds, followed by a drying step, usually under ambient room conditions (Figure 1B – D). The storage and transport of the dried tips are recommended in zip-locked bags with desiccant bags [19,20]. Before the analysis, the polymer head of the Mitra tip might be separated, and analytes are extracted with the proper extraction solvent (e.g. methanol, water, acetonitrile, or a mixture of them) [21]. Moreover, the extraction step could also be done in a 96-well autorack and using the liquid handling robots so that the process might be automated [22].

The collected blood volume is relatively small; thus, the analytical method must be sensitive enough to detect and quantify analytes of interest. Due to the high degree of specificity, sensitivity, and selectivity, the use of liquid chromatography-mass spectrometry (LC-MS) instrumentation is the most popular and suitable technique for analyzing micro volumes of analytes [23]. During the method development, the extracts of Mitra tips might be pre-treated by a procedure like protein precipitation, solid phase extraction, or liquid-liquid extraction methods. The selection of extraction solvent and sample preparation technique is essential as it affects the reduction of matrix effects and extraction recoveries of analytes [24].

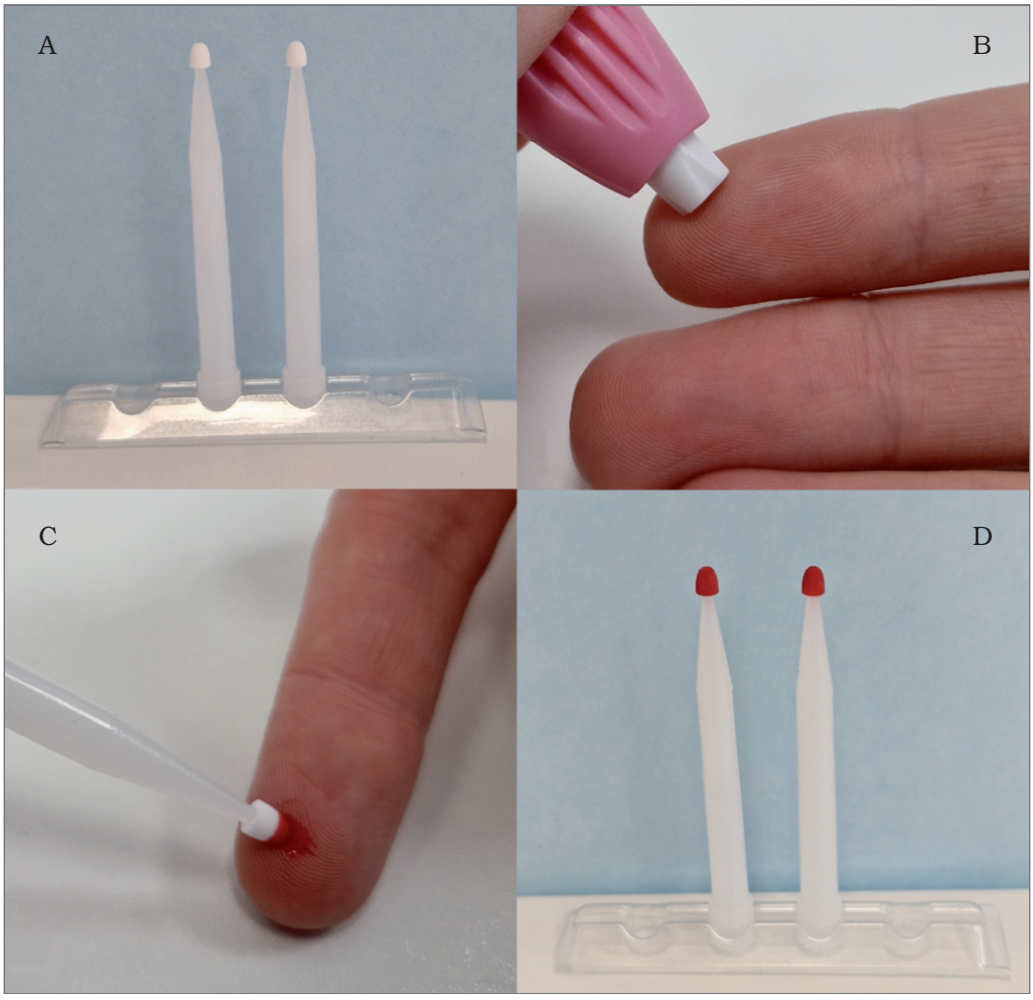

Fig. 1 Illustration of Mitra tip (A) and workflow of blood sampling (B – D)

The main advantage of VAMS is that it allows hematocrit-independent sample collection with accurate and reproducible blood volumes. However, some authors observed the impact of hematocrit on the extraction efficacy using VAMS [20, 25]. Consequently, optimizing the extraction parameters focusing on the hematocrit range during the method development is recommended. So far VAMS technique has been used in various clinical and nonclinical studies such as pharmacokinetic studies, TDM examinations, or metabolomic studies [16]. The drugs suitable for VAMS microsampling should possess good stability and solubility in water or organic solvents. An example of drugs ideal for TDM using VAMS sampling is summarized in Table 1.

Table 1 Examples of drugs applicable for monitoring using the volumetric absorptive microsampling technique [17]

Drug class	Analytes
Antibiotics	cefepime, fosfomycin, linezolid, meropenem, tazobactam, vancomycin
Anticonvulsants	brivaracetam, carbamezpine, ethosuximide, lamotrigine, levetiracetam, phenytoin, rufinamide, topiramate, valproic acid
Immunosuppressants	cyclosporin A, everolimus, mycophenolic acid, tacrolimus, sirolimus
Cardiovascular drugs	acetylsalicylic acid, atenolol, lisinopril, simvastatin, valsartan
Endogenous compounds	HbA1c, gamma-hydroxybutyric acid

In addition, the introduction of the VAMS technique in routine TDM enables to increase in the adherence of the patients to the therapy, as it allows home sampling without the assistance of trained personnel. Dried Mitra sticks can be delivered to the laboratory, packed in envelopes, and sent via standard postal services [26]. Moreover, several authors using the questionnaire forms confirmed that VAMS home sampling is preferred over traditional blood sampling in the case of routine monitoring [27, 28].

Direct oral anticoagulants

Oral vitamin K antagonist anticoagulants have been used for long-term treatment and prevention of thromboembolic events in many indications for decades [29]. However, due to unpredictable pharmacokinetics and various aspects influencing the efficacy of therapy

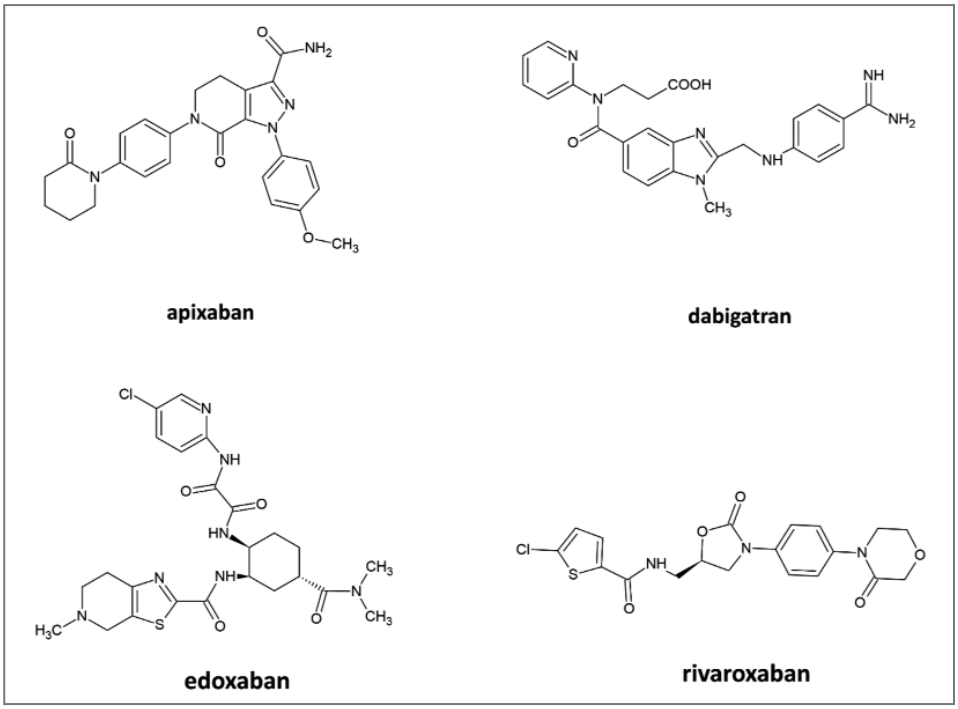

Fig. 2 Chemical structures of direct oral anticoagulants

(mainly genetic factors and food/drug interactions), safer and more effective drug development has arisen [30]. Since 2008, several direct oral anticoagulants (DOACs) have been approved in the EU as a safer alternative to warfarin in avoiding embolic complications. Currently, four DOACs are available in the EU (Figure 2), including direct thrombin inhibitors (dabigatran, the active molecule of prodrug dabigatran etexilate) and direct inhibitors of factor Xa (apixaban, edoxaban and rivaroxaban) [31]. This group of anticoagulants has a rapid onset and offset of action, fewer drug and food interactions, a wider therapeutic window, and a more predictable pharmacological effect than vitamin K antagonists [32]. The basic pharmacokinetics features and drug characteristics of DOACs are summarized in Table 2.

One of the main advantages over vitamin K antagonists is that DOACs do not require routine biochemical monitoring and dose adjustment. However, their relatively short plasma halftime and once-daily dosing (edoxaban and rivaroxaban) may decrease patients' adherence to therapy and strongly impact the therapeutic effect [33]. In addition, all of them are metabolized by cytochrome-P450 enzymes, predominantly by CYP3A4 isoform, and interact with P-glycoprotein and breast cancer resistance protein (BCRP). As a consequence, the coadministration of drugs affecting the above-mentioned enzymes and transporters may impact the therapy efficacy. Dose adjustment of DOACs is suggested in some clinical situations like renal failure and during the therapy of extremely obese or thin and elderly patients [34]. As we can clearly observe a growing number of patients taking DOACs, the monitoring of plasma levels might be beneficial and even mandatory in some circumstances. Unfortunately, there is no exact definition of the recommended therapeutic range despite the apparent relation between reached plasma levels and clinical effects [35]. In 2018 International Council for Standardization in Haematology reported recommendations for laboratory measurement of DOACs, where they proposed expected plasma concentrations in patients treated with DOACs in the prevention of stroke, pulmonary embolism, and venous thromboembolism. Nonetheless, it should be mentioned that reference intervals are mainly established on "on-therapy" concentrations [36].

Table 2 Selected pharmacokinetics characteristic of direct oral anticoagulants

	Dabigatran	Apixaban	Edoxaban	Rivaroxaban
Target	free and clot-bound thrombin	factor Xa	factor Xa	factor Xa
Prodrug	yes	no	no	no
Bioavability	3 – 7%	50%	60%	80 – 100%
Protein binding	35%	85%	55%	90-95%
Dominant clearance	renal	hepatobiliary	hepatobiliary	hepatobiliary
T_{max}	1.5 – 3 hrs	3 – 4 hrs	1 – 2 hrs	2 – 3 hrs
Dosing frequency	twice daily	twice daily	once daily	once daily

Several specific coagulation assays are established in routine laboratory assessments to monitor plasma levels and perform the TDM of DOACs. They are based on diluted thrombin time, ecarin clotting time, or drug-specific chromogenic anti-Xa tests. However, several disadvantages should be noticed, such as calibration to the particular drug, the imprecise results at low plasma levels, and none of the methods being specific enough for quantifying all DOACs in a single run [37]. Combining liquid chromatography with tandem mass spectrometry

(LC-MS/MS) introduces a unique method for measuring DOACs in biological samples. Up to now, several LC-MS/MS methods have been reported to determine DOACs simultaneously in human plasma samples [38–44]. In our laboratory we are finishing the validation of the LC-MS/MS method, allowing high-throughput analysis suitable for routine monitoring. A one-step extraction procedure in 96-well formate was used for sample preparation, enabling to process and analyze more than 80 samples per a working day.

The potential of volumetric absorptive microsampling in DOACs monitoring

In many indications, direct oral anticoagulants are more often prescribed over vitamin K antagonists. As it was mentioned above, the monitoring of plasma levels might be beneficial in some special situations [1]. Up to now, only one paper has been published for the determination of all DOACs simultaneously using the microsampling technique. Foerster et al. developed and validated LC/MS-MS method using DBS sampling to determine apixaban, edoxaban, rivaroxaban, and dabigatran [45]. Considering the physiochemical properties of DOACs (Table 3), VAMS seems to be a suitable sampling method and might be another possible choice for blood collection. Implementing Mitra stick for LC-MS analysis could help introduce routine TDM of DOACs in broader patient populations. During method development for DOACs determination in our laboratory we also did preliminary experiments using Mitra sticks. Three extraction solvents were evaluated (1% formic acid in 50% methanol, 1% formic acid in methanol, and methanol) and all of them were capable of extracting all analytes of interest. However, using pure methanol we yielded the highest extraction recovery (data not shown). The aim of our future study will be the implementation of the VAMS technique for the determination of DOACs using the already developed LC-MS/MS method. We will focus on the optimization of extraction procedures (extraction solvent composition, sonication of extracts, time and temperature of extraction), stability of analytes during different storage conditions, and comparison of plasma levels against blood levels.

Table 3 Physiochemical properties of direct oral anticoagulants

	Molecular mass (Da)	Lipophilicity (LogP)	pKa	PSA (Å)
Apixaban	459.49	2.33	pKa^a = 13.12	110.77
Dabigatran	471.52	2.37	pKa^a = 11.51 pKa^b = 4.24	150.22
Edoxaban	548.06	1.61	pKa^a = 11.08 pKa^b = 7.23	136.62
Rivaroxaban	435.88	1.74	pKa^a = 13.6	88.18

LogP, octanol/water partition coefficient; pKa, dissociation constant; PSA, polar surface area.
[a] acid function, [b] basic function.

CONCLUSION

Although DOACs laboratory monitoring is not routinely necessary, plasma-level quantification might be helpful for clinicians to avoid thromboembolic events or bleeding associated with an inappropriate pharmacokinetic profile in an individual patient. Microsampling devices for biological fluids collection are still growing alternatives to classical methods, such as venipuncture. This new approach, like volumetric absorptive microsampling, has the potential to be applied in routine therapeutic drug monitoring due to advantages like home performance and ease of sampling by the patient itself, simplified workflows, and cost

savings. We believe that volumetric absorptive microsampling using a commercially available device, Mitra stick, could be a promising tool for introducing routine therapeutic monitoring of direct oral anticoagulants therapy.

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CLINICO-ETIOLOGICAL AND CLINICO-HAEMATOLOGICAL STUDY OF PATIENTS WITH PANCYTOPENIA IN RURAL AREA

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Abstract

Background: Pancytopenia is a manifestation of other underlying conditions, commonly associated with multiple benign and malignant conditions. Any patient presenting with pancytopenia requires a thorough evaluation to identify the underlying aetiology.

Aim: This current evaluates various hematological parameters including bone marrow aspiration (where ever feasible) in pancytopenia in adult group. Study also correlates clinico-haematological profile.

Method: Fifty patients with a hematological diagnosis of pancytopenia were studied during the period August 2020 to August 2022. The study included adult patients of both sexes having the age of 18 years and above. Tests for complete blood count, reticulocyte count, peripheral smear, bone marrow aspiration, and trephine biopsy were done.

Results: Various etiological factors were identified in which majority were of megaloblastic anaemia (48.9%), followed by mixed nutritional anaemia (22.2%), hypersplenism (13.3%), aplastic anaemia (8.9%), malignant conditions (6.7%), myelodysplastic syndromes (2%), and others (4%) respectively. Megaloblastic anaemia cases observed in the age group of 31–50 years with male preponderance. Hemoglobin, TLC, Platelet count, and Reticulocyte count ranged from 2 g% - 10g%, 500–4000 cells/cumm, 24,000–1.5 lakh cells/cumm, and 0.1% – 2%. MCV was higher than 100 fl in 57.5% of cases. Majority of the patients had macrocytic and dimorphic anaemia. Hypersegmented neutrophils were present in all the patients. Bone marrow of Megaloblastic anaemia was hypercellular. Megaloblastic erythropoiesis with giant meta- myelocytes and band forms were seen. Nutritional anaemia seen in the age group of 51-60 years. Haemoglobin, TLC, Platelet count, and Reticulocyte count ranges from 2.3 g%–7.8 g%, 1000–4000 cells/cumm, 5000–1.4 lakh cells/cumm, and 0.1–8%. Two cases had microcytic hypochromic anaemia in Nutritional anaemia. Bone marrow was hypercellular with a reversal of M:E ratio in 93.8% of cases. In hypersplenism seen in the age group of 51–60 years. Haemoglobin, TLC, Platelet count, and Reticulocyte count ranges from 3.8 g% – 10 g%, 1700–3800 cells/cumm, 26000-1.4 lakh cells/cumm, and 0.6–2% respectilvey in hypersplenism. 40% of hypersplenism patients had microcytic hypochromic anaemia. Bone marrow was hypercellular with a reversal of M:E ratio in 70% of hypersplenism cases. Aplastic anaemia seen in the age group of 41–50 years. Haemoglobin, TLC, Platelet count, and Reticulocyte count ranges from 3.1–10 g%, 1100–4000 cells/cumm, 51000–1.5lakh cells/cumm, and 0.2%–1.8%. Aplastic anaemia (35.8%) cases showed macrocytosis. Bone marrow was hypocellular with an increase in marrow fat and Lymphocytes and plasma cells were prominent in Aplastic anaemia cases. Leukaemia commonly seen in the age group of 31–40 years with male predominance. Hemoglobin, TLC, and Reticulocyte count ranges from 5.1–9.8%, 1100–4000 cells/cumm, and 0.6–2% respectively. Bone marrow was hypercellular with a reversal of M:E ratio in 80%.

Conclusion: Megaloblastic anaemia was the commonest cause of pancytopenia. Most other studies have reported aplastic anaemia as the commonest cause. This seems to reflect the higher prevalence of nutritional anaemia in the Indian subjects. The haematological parameters and bone marrow morphological features in patients with megaloblastic anaemia, aplastic anaemia, and malignant diseases including MDS in the present study were comparable to the findings by other authors. Uncommon etiological factors like dengue fever and hemolytic anaemia were identified in this study. A comprehensive clinical, haematological, and bone marrow study of patients with pancytopenia usually helps in identification of the underlying cause. However, in view of a wide array of etiological factors, pancytopenia continues to be a challenge for hematologists.

Keywords: Anaemia, Reticulocyte count, Platelet count, Pancytopenia.

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INTRODUCTION

Pancytopenia is characterized by a hemoglobin value of less than 12 g/dL in women and 13 g/dL in men, platelets of less than 150,000 per μL, and leukocytes of less than 4000 per ml (absolute neutrophil count of less than 1800 per ml).[1] Pancytopenia is a common clinico-hematological entity encountered in day-to-day clinical practice.[2,3] Pancytopenia refers to a disorder in which all three elements of the blood (RBCs, WBCs, and Platelets) are lower in counts than normal. Thus, it is not a disease entity by itself, but rather a triad of findings. It is a primarily or secondarily affecting bone marrow manifesting and lead to various hematological derangements, which is reflected in the peripheral blood smear as pancytopenia. There are varying trends in its aetiology, clinical pattern, treatment modalities, and outcome in different studies.[4-7]

Pancytopenia is defined as hemoglobin < 12 gm%, WBCs count < 4 x 10^9/L, and platelet count < 100 x 10^9/L. Anaemia defined as mild (Hb 9–12 gm%), moderate (Hb 5–9gm%), severe (Hb 3,000/ mm3), moderate (WBCs 1,000-3,000/mm3), and severe (WBCs 50,000/mm3), moderate (platelet count 20,000-50,000/mm3) and severe as (platelet count < 20,000/mm3). Most of the time pancytopenia is insidious in onset. The presenting symptoms are usually anaemia and thrombocytopenia, Leukopenia is an uncommon cause of initial presentation. There are many factors encompassing geographic distribution and genetic disturbances which cause pancytopenia according to various studies.[6,7]

To understand the aetiology of pancytopenia, bone marrow biopsy plays a significant role. In some other selected cases radiological, biochemical, and microbiological investigations are useful. The severity of pancytopenia and underlying aetiology determine management and prognosis. Thus, identification of correct cause will help in treatment. This study is therefore aimed to identify the frequent causes of pancytopenia in patients presenting to a rural area. There are blund to be variations in causes leading to pancytopenia. Similar studies are available in literature; an attempt is being made to correlate with regards to rural population.

Although it is a common clinical pattern with an extensive differential diagnosis, there is little discussion of this abnormality in major textbooks of internal medicine and hematology. Since the underlying pathology of pancytopenia determines the management and prognosis of patients, there is a definite need to study about pancytopenia.

The aim of the study was to evaluate various hematological parameters including bone marrow aspiration (where ever feasible) in pancytopenia in adult group. The study also correlates clinico-haematological profile.

MATERIALS AND METHODS

Fifty patients with a hematological diagnosis of pancytopenia were studied during the period, august 2020 to August 2022, in the Department of Pathology, SVS Medical College and hospital, Mahabubnagar.

Inclusion criteria: Patients within the age group of 18–60 with hematological evidence of pancytopenia.

Exclusion criteria: Patients below 18 years and above 60 years with pancytopenia. Patients not amenable for follow up were excluded.

Leishman's Stain was done by observing Cytoplasm in pink and Nucleus in blue color.

Periodic acid Schiff staining was done according to standard laboratory procedures

Counter stained with Harris' hematoxylin for 5–10 minutes. Nuclei in Blue color, substances showing positive reaction had Magenta Pink.

Clinical history recording and examination of all the identified cases of pancytopenia were done as per proforma. Two ml of anticoagulated blood was collected for complete hemogram.

Sudan black and Perls' stains were used wherever indicated.

Bleeding time measured by Ivy's method, and Clotting time by Tube method. While Hemoglobin percentage, Total leucocyte count, RBC Count, Platelet count, and Packed cell volume were measured by Sysmex cell counter.

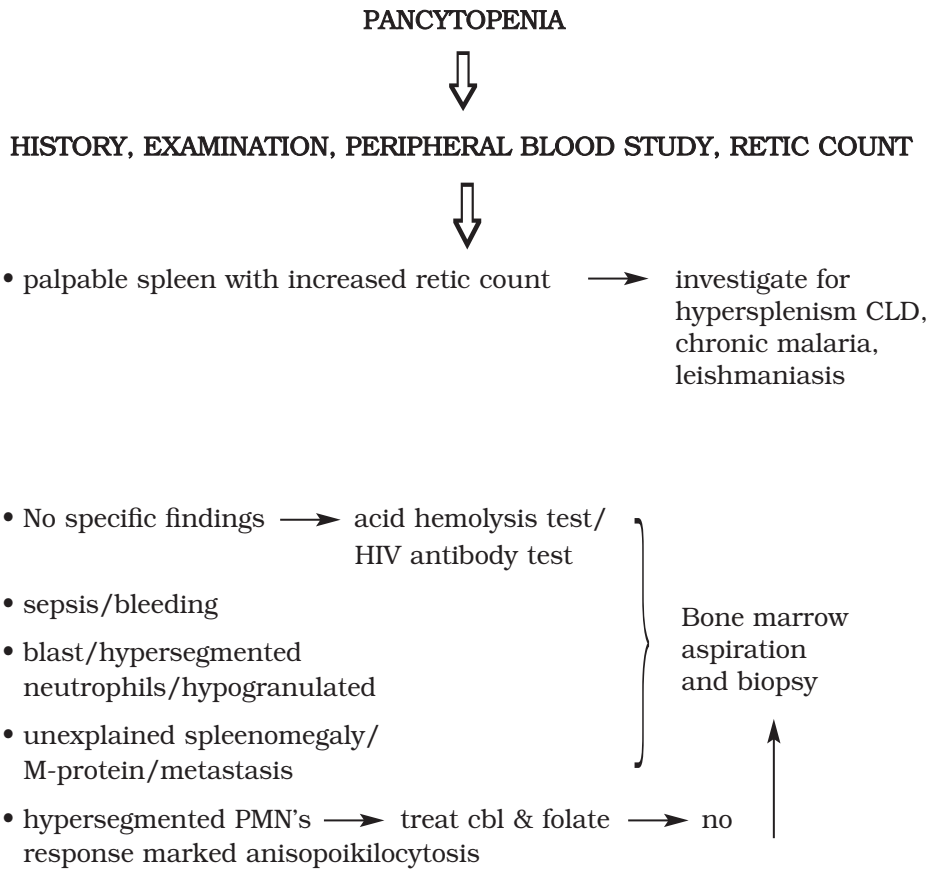
The peripheral smear was studied after staining with Leishman's stain. Special stains – Periodic acid Schiff reagent stain, Myeloperoxidase, Sudan black and Perls' stains were used wherever indicated.

Estimation of Serum Ferritin done by Chemiluminescence immunoassay (Lilac Acculite CLIA).

Bone marrow aspiration was done in all the patients to identify the aetiology. Slides were stained with Leishman's stain.

Hypochromicity on smear with low ferritin and MCV<75fL is called as Iron Deficiency Anaemia. Iron in the control subjects were 118.37 ± 20.79 µ/dl.

Iron deficiency anaemia is defined as Hb less than 11g/dl with the presence of a mean corpuscular volume (MCV) of less than 70 fl and serum ferritin below 30 ng/ml (in presence of infections).



Statistical analysis: The Crosstabs procedure forms two-way and multiway tables and provides a variety of tests and measures of association for two-way tables. The structure of the table and whether categories are ordered determine which test or measure to use. The data were tabulated in Microsoft Office Excel and analyzed using SPSS software version

18.0 (SPSS Inc, US). The chi-square test was used to analyze the association between two categorical variables. P value of <0.05 was statistically significant.

RESULTS

Most of the patients were in the age group of 41-60 years (53%). The sex distribution of pancytopenia showed a male preponderance. The male to female ratio was 2.1:1.

Generalised weakness (86%) was the commonest symptom in pancytopenic patients, followed by fever (39%), bleeding manifestations (5%), and pain abdomen (24%).

Totally 8% of the patients had Hb values between 1-3 g, followed by 22% were of 3.1 to 5 g of Hb levels, and 52% were of 5.1 to 7 g Hb levels.

Total leukocyte count was in the range of 500-4000 cells/mm^3. Most (40%) of the patients had values in the range of 3100-4000 cells/mm^3. 8% of the patients had values between 500 and 1000 cells/mm^3.

Most (36%) patients had platelet counts in the range of 51,000–75,000 cells/ mm^3. 24% of patients had platelet counts in the range of 4000– 50000 cells/mm^3.

The reticulocyte count varied from 0.1–20%. Majority (88%) of the patients had reticulocyte count in the range of 0.1–2%, and 4% of patients were between 6.1 to 20.

Bone marrow aspirate analysis shows various types of cellularity, such as 8% of hypocellularity, 80% of hypercellularity, and 12% of normocellular respectively.

a. **Pancytopenia associated with hypocellular marrow**: In the present study, 10 out of 50 patients had hypocellular marrow. The causes were varied, 40% being belonging to hypcellular marrow and Aplastic anaemia, which showed a peak incidence (60%, n=3) in the age group (41–50 years). The least incidence (20%, n=1) was seen in the age group of 31–40 years. Dengue fever (20%) and hypersplenism (20%) contributed to rest of the cases of hypocellular marrow. Aplastic anaemia (autoimmunity) was more common in males (60%, n=3). The male to female ratio of incidence was 1.5:1. The haemoglobin percentage varied from 3.1–10 gm%. Most patients (40%, n=2) had hemoglobin values in the range of 3.1-5 g%. 20% of patients were seen to have hemoglobin values in the range of 7.1–10 g%. The total leukocyte count ranged from 1100–4000 cells/mm^3. Most patients (40%, n=2) had a value in the range of 1100–2000 cells/ $mm^3$and 3100-4000 cells/mm^3. The reticulocyte count was in the range of 0.1–1.5%. Majority of the patients (80%,n=4) had values in the range of 0.1–1.0%. Most of the patients (64.2%) had normocytic normochromic erythrocytes. Some (35.8%) showed macrocytosis. 42.8% of them had relative neutrophilia while the others had relative lymphocytosis. The erythrocyte sedimentation rate was increased in most of the patients and ranged from 28–140 mm/hr. The bone marrow was hypocellular and the aspirate was mostly composed of fat cells. Other precursors appeared normal. There was a relative increase in the number of plasma cells and lymphocytes.

b. **Pancytopenia with hypercellular and normocellular marrow:** Hypercellular bone marrow was observed in 38 patients and it was normocellular in 7 patients. Most common etiology noted was megaloblastic anaemia (48.9%, n=22), followed by nutritional anaemia (22.2%, n=10), hypersplenism (13.3%, n=5), leukaemia (6.7%, n=4), myelodysplastic syndromes (2.2%, n=1), and others. Normocellular marrow was seen in (8%) megaloblastic anaemia, (4%) hypersplenism, and 2% in nutritional anaemia. There were 10 Hypocellular marrow, and 30 hypercellular marrows with pancytopenia were recorded.

Table 1 Depicting various categories and cellularity

CATEGORY	Number of cases	% of cases	Hypercellular marrow: 30 Percentage %	Hypocellular Marrow: 10 Percentage %	Normocellular Marrow: 10 Percentage %
Megaloblastic marrow	24	48	80	0	0
Mixed nutritional (dimorphic)	10	20	0	0	100
hypersplenism	2	4	0	20	0
MDS	1	2	3.3	0	0
MM	1	2	3.3	0	0
Acute leukaemia	3	6	10	0	0
Hemolytic anaemia	1	2	3.3	0	0
Dengue fever	2	4	0	20	0
aplastic	4	8	0	40	0
unclassifiable	2	4	0	20	0

Pancytopenia with megaloblastic anaemia: In the present study, 24 cases of megaloblastic anaemia were seen. It constituted 48% of cases of pancytopenia and 80% of all patients with cellular marrow. Megaloblastic anaemia was observed in the age group ranging from 21–60 years. Majority were seen in the age group of 31–50 years (63.6%, n=14). There were male preponderance and the male to female ratio was 2.4:1. Majority of the patients (68.2%, n=15) had values in the range of 5.1–7gm%. Majority of the patients (81.8%, n=20) had a leukocyte count in the range of 2100-4000 cells/mm^3. 4.6% of patients had values ranging from 500–1000 cells/mm^3. Most of the patients (40.9%) had a platelet count in the range of 51,000–75,000 cells/mm^3. 9.1% patients had values in the range of 26,000–50,000 cells/mm^3. The reticulocyte count varied from 0.1–6%. Most of them (77.3%) had reticulocyte count in the range of 0.1–2%.

Macro ovalocytosis with a considerable degree of anisopoikilocytosis were the main features. Mean corpuscular volume was more than 100 fl in 57.5% of patients. Dimorphic blood picture was seen in 10 patients (30%). Hypersegmented neutrophils were seen in most of the patients. Basophilic stippling and cabot rings were present. Platelets were reduced in number in all the cases.

The bone marrow was hypercellular with a reduction of fat cells in most of the patients (81.8%). Four patients (18.2%) had a normocellular marrow. Erythroid hyperplasia with megaloblastic maturation and reversal of M:E ratio was seen in all the patients. Megakaryopoiesis was normal in 63.6%, decreased in 18.2%, and increased in 18.2% of patients.

Pancytopenia with mixed nutritional anaemia

In the present study, 10 patients (20%) of pancytopenia were observed to have nutritional anaemia as the etiology, which contributed to all cases of normocellular marrow.

Nutritional anaemia was seen in the age group of 18–60 years. Majority of the patients (40%) were in the age group of 51–60 years. 20% of them were in the age group of 21–40 years. There was a male predominance and the male to female ratio of incidence was 2.3:1.

Hemoglobin percentage varied from 2.3–7.8%. Majority of the patients (50%) had haemoglobin in the range of 3.1–5 gm%.

The total leukocyte count ranged from 1000–4000 cells/ mm^3. Most of the patients (60%) had a value in the range of 3100–4000 cells/ mm^3. 10% of the patients had a count of 500–1000 cells/ mm^3. Most of the patients (40%) had a value between 51,000 and 75,000 cells/mm^3. The reticulocyte count varied from 0.1-8%. In majority of the patients (50%) the values ranged from 0.1–2%. Most of the patients had normocytic normochronic anaemia. In two patients had microcytic hypochromic anaemia, MCV was in the range of 65.6–108 fl.

MCHC was in the range of 26–34.7% and MCH was in the range of 18–38.9 pg. Nine patients (90%) had hypercellular marrow with a reversal of M:E ratio. Erythroid hyperplasia along with both megaloblastic and micronormoblastic maturation was observed in all the patients. Leucopoiesis was normal. Megakaryocytes were either normal or increased. Only two patients had decreased megakaryopoiesis.

Pancytopenia with hypersplenism

In the present study, 2 patients of hypersplenism were seen, which constituted to 4% of cases with pancytopenia and 20% of all hypocellular marrows. Hypersplenism was seen to occur in the age group ranging from 41–60 years. Majority of them were in the age group of 51–60 years. There was a male preponderance and male to female ratio of incidence was 2:1. Hemoglobin percentage varied from 3.8–10 gm%. Majority of the patients (50%) had values in the range of 7.1–10 gm%. The total leukocyte count ranged from 1700-3800 cells/mm^3. Majority of the patients (50%) had values between 1100–2000 cells/mm^3. The reticulocyte count ranged from 0.6–2%. Majority (50%) of the patients had values in the range of 0.6–1%. Most of the patients (60%) had normocytic normochromic anaemia. 40% of them had microcytic hypochromic anaemia with a severe degree of anisopoikilocytosis. MCV was in the range of 56.8 fl – 94.6 fl. 66.7% of patients had hypercellular marrow while the rest had normocellular (33.3%). Erythroid hyperplasia was observed in 90% of patients. Leukopoiesis was normal in 90% patients. Megakaryopoiesis was normal in 60% of patients, while it was increased in the rest.

Pancytopenia with malignant diseases, myelodysplastic syndromes, and others

In the present study, 10% of the patients with pancytopenia had the following etiologies of acute leukaemia (3, AML category), one case of multiple myeloma. They accounted to 10% of cases of pancytopenia with cellular marrow. The age ranged from 21–40 years. Majority of them (66.7%) were in the age group of 31–40 years. There was a male predominance. The male to female ratio of incidence was 2:1. The hemoglobin percentage ranged from 5.1–9.8%. 66.7% of the patients had values between 7.1 and 10 gm%. The total leukocyte count varied from 1100–4000 cells/mm^3. 66.7% of the patients had values between 3100–4000 cells/ mm^3. Platelet count ranged from 26,000-89,000 cells/mm^3. The reticulocyte count ranged from 0.6–2%. 66.7% of the patients had a value ranging from 0.6–1%. These patients presented with peripheral pancytopenia. The erythrocytes were normocytic normochromic. The leukocyte count was decreased with a presence of immature cells. Myeloblasts with fine chromatin, 2-3 nucleoli, and occasional Auer rods were seen. Platelets were decreased. Bone marrow was hypercellular in 100% of the patients. Erythroid series were decreased in 40% of the patients and increased in 60% patients. Myeloid hyperplasia was observed with more than 30% blasts in all of them. Immunocytochemistry demonstrated MPO positivity in them. Megakaryocytes were reduced.

One case of myelodysplastic syndrome was observed in the present study in a female patient aged 55 years who had peripheral pancytopenia with a macrocytic type of anaemia.

The aspirate was hypercellular with erythroid hyperplasia and features of dyserythropoiesis which included megaloblastic erythroblasts, nuclear fragmentation, budding multiple nuclei, and hyperlobulation. Dysmyelopoiesis with hypogranular neutrophils and blasts were also observed. Large hypolobulated megakaryocytes were seen.

Dengue fever

Two cases of dengue fever in an 18-year-old male and a 22 year old female presented with pancytopenia. The total leukocyte count was 800 cells/mm^3 and platelet count was 28,000 cells/cumm. Bone marrow was hypercellular with erythroid hyperplasia and a reversal of M:E ratio.

Hemolytic anaemia:

One case of hemolytic anaemia presented with pancytopenia. The patient was a 21-year-old female. The peripheral blood showed features of hemolysis. Erythrocytes showed a moderate degree of anisopoikilocytosis with increased polychromatophilic RBCs. The reticulocyte count was markedly increased. The bone marrow was hypercellular with a reversal of M:E ratio. Erythroid hyperplasia with normoblastic maturation was seen. Megakaryocytic hyperplasia was also noted.

Table 2 Association between Pancytopenia etiology and Haematological Parameters

	Hb % (gm/dl)	TLC (cells/mm^3)	Platelet (cells/mm^3)
Megaloblastic anaemia associated with pancytopenia	2–10	500–4000	26000–1,50,000
Nutritional anaemia (mixed)	2.3–7.8	1000–4000	5000–1,40,000
Aplastic anaemia associated with pancytopenia	3.1–10	1100–4000	5100–1,50,000
Hypersplenism	3.8–10	1700–3800	26,000–1,40,000
Leukaemia associated with pancytopenia	5.1–9.3	1100–4000	26000–89000

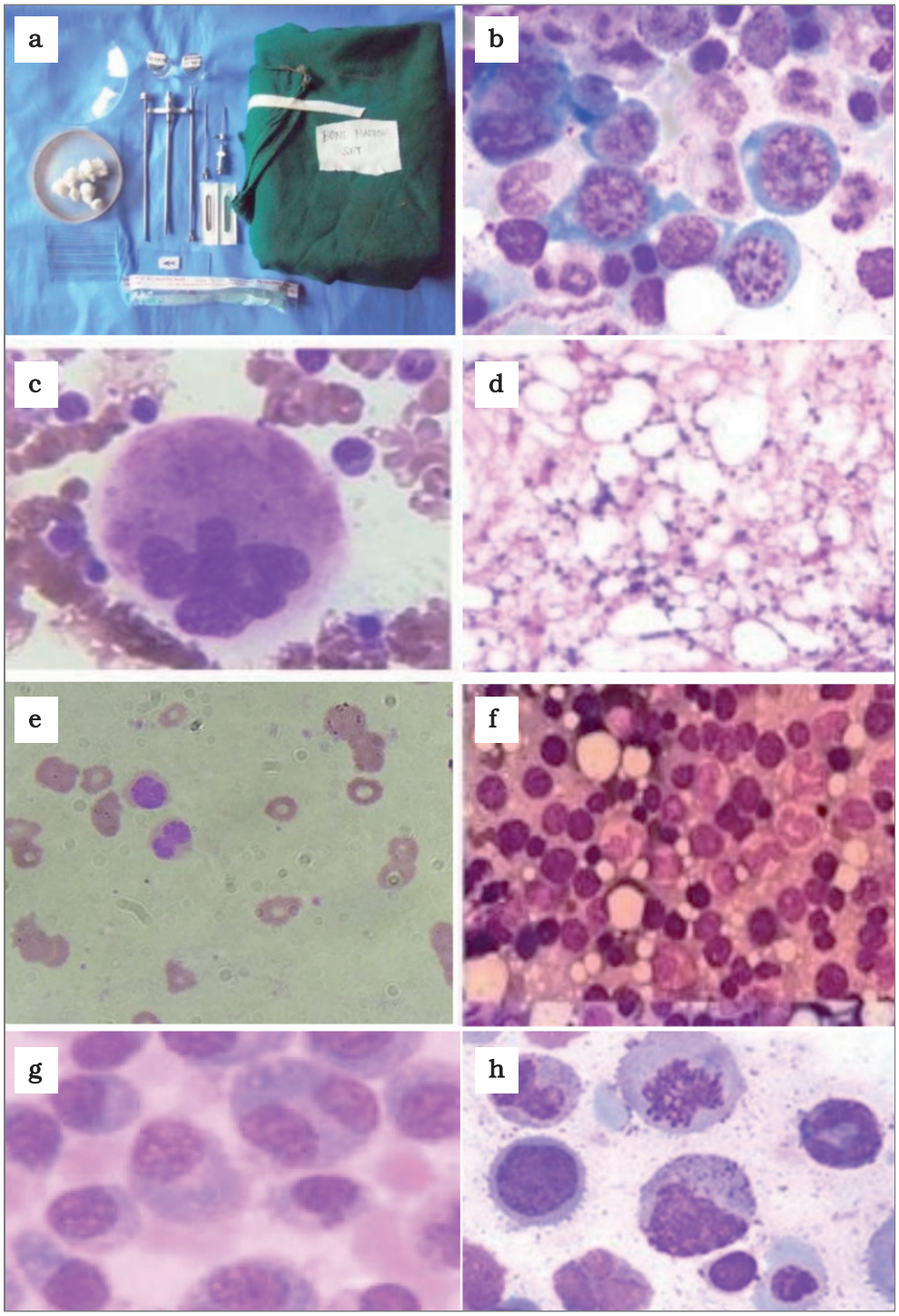


Fig. 1 a. Bone Marrow Set. b. Erythroid Hyperplasia (100x). c. Megakaryocytes (100x). d. Aplastic anaemia (100x). e. Microcytic Hypochromia with Neutrophils (100x). f. Erythroid Hyperplasia (100x). g. Plasma Cells (100x). h. Megaloblasts with Mitosis (100x).

DISCUSSION

In the present study, megaloblastic anaemia (48.9%) was the commonest cause of pancytopenia, followed by mixed nutritional anaemia (22.2%), hypersplenism (13.3%), aplastic anaemia (10%), malignant diseases (5%), myelodysplastic syndromes (0.6%), and other (4%) uncommon causes like Dengue fever (2%) and Hemolytic anaemia (2%).

The commonest cause of pancytopenia reported from various studies throughout the world has been aplastic anaemia (Table 3).

This is in contrast with the results of the present study where the commonest cause of pancytopenia was megaloblastic anaemia. This seems to reflect the higher prevalence of nutritional anaemia in Indian subjects as well as in developing countries. However, similar results have been reported in studies from other Indian centres.

Table 3 Causes of pancytopenia in various studies

Study	Country	Year	No. of cases	Commonest cause	Second most common cause
Retief FP, Heyns AD[8]	South Africa	1976	195	Bone marrow failure (67.7%)	Severe infection (9.7%)
Imbert M et al[9]	Europe	1989	213	Malignant myeloid disorders (42%)	Malignant lymphoid disorders (18%)
Varma N, Dash S[10]	India	1992	202	Aplastic anaemia (40.6%)	Megaloblastic anaemia (23.26%)
Tilak V, Jain R[5]	India	1998	77	Megaloblastic anaemia (68%)	Aplastic anaemia (7.7%)
Khodke et al.[11]	India	2001	166	Hypoplastic anaemia (29.51%)	Megaloblast ic anaemia (22.3%)
Naeem Khan M et al.[12]	Pakistan	2001	30	Aplastic anaemia (20%)	Megaloblastic anaemia (16.7%)
Kumar R et al.13	India	2001	166	Aplastic anaemia (29.5%)	Megaloblastic anaemia (22.3%)
Osama Ishtiaq et al.[14]	Pakistan	2002	100	Megaloblastic anaemia (39%)	Hypersplenism (19%)
Mobina et al.[15]	Pakistan	2005	392	Megaloblastic anaemia (35.95%)	Hypersplenism (16.3%)
Jha et al.[16]	Nepal	2008	148	Hypoplastic anaemia (29.5%)	Megaloblastic anaemia (23.64%)
Varma A et al [17]	India	2018	251	Megaloblastic anaemia (48.5%)	Dimorphic anaemia (17.8%)
Present study	India	2022	50	Megaloblastic anaemia (48.9%)	Nutritional anaemia (22.2%)

Megaloblastic anaemia associated with pancytopenia

Megaloblastic anaemia is common in India. This seems to reflect the higher prevalence of nutritional anaemia in Indian subjects. In a study of pancytopenia cases by Jha et al., the age range was 10–79 years (31 years). There was a male preponderance and male to female ratio was 1.5:1. In the study by Kumar et al.[13] the ages ranged from 14–73 years (39.5%). There was a female preponderance and the male to female ratio was 2:1. In the present study, the age ranged from 18–70 years. Majority of the patients were in the age group of 31–50 years (62%). There was a male preponderance and the male to female ratio was 2.4:1.

The principal hematologic manifestations are varying degrees of anaemia, leucopenia, thrombocytopenia, anisopoikilocytosis, macroovalocytosis and hyper-segmented neutrophils.

In a study by Kishore Khodke et al.[11] 20/22 cases showed anisocytosis, 10/22 cases showed dimorphic blood picture, and 20/22 cases showed hypersegmented neutrophils. In the study by Tilak et al.[5] 51/53 cases showed anisocytosis, 45/53 cases showed hypersegmented neutrophils, 13/53 showed circulating erythroblasts. Reticulocytes were seen in 5/53 and relative lymphocytosis was seen in 7/53 cases.

In the present study, macroovalocytes with considerable degree of anisopoikilocytosis were the main features in all the cases. MCV was more than 100 fl in 57.5% of cases and dimorphic blood picture was seen in 30% of cases (10 patients). Hypersegmented neutrophils were seen in most of the patients.

Bone marrow is usually hypercellular with predominantly megaloblastic erythropoiesis. Giant band forms, metamyelocytes, and giant megakaryocytes are also seen. In the present study, the bone marrow was hypercellular with a reduction of fat cells in most of the patients (81.8%). Four patients (18.2%) had normocellular marrow. Erythroid hyperplasia with megaloblastic maturation was seen in all the patients.

Nutritional anaemia (mixed)

Nutritional as a common etiological factor causing pancytopenia is well recognized and established. The nutritional deficiency of either B_{12} or folate results in megaloblastic anaemia. Other causes include mixed deficiency anaemia (microcytes and macrocytic). Mobina et al.[15] in their study of 392 cases of pancytopenia found 11.2% cases of mixed deficiency anaemia.

In the present study, mixed deficiency was seen in 22.2% of patients. This percentage is much lower than expected because 60–80% of world population is affected by iron deficiency anaemia which is the most common preventable nutritional deficiency in the world. The possible explanation is that majority of the cases present with anaemia rather than pancytopenia and are diagnosed on smear examination and treated as outpatients.

The age ranged in the present study from 18–60 years. There was a male preponderance and male to female ratio was 2.3:1. Most of the patients had dimorphic anaemia. Two patients had macrocytic and hypochromic anaemia. Bone marrow was hypercellular. Erythroid hyperplasia with both megaloblastic and micro normoblastic maturation was observed in all the patients. Leucopoiesis was normal. Megakaryopoeisis was either normal or increased.

Aplastic anaemia associated with pancytopenia

In the study by Kumar et al. the ages ranged from 12–63 years (29 years). There was a male preponderance and male to female ratio was 1.4:1. In the study by Jha et al.[16] the ages ranged from 1.5–70 years (17 years). There was a male preponderance with male to female ratio of 1.3:1.

In the present study, the ages ranged from 31-60 years. Majority of the patients were in the age group of 41–50 years. Aplastic anaemia was more common in males. The male to female ratio was 1.5:1.

In the study by Kishore Khodke, 3/7 patients showed anisocytosis and 1/7 patients showed relative lymphocytosis. In the study by Tilak et al. 2/6 patients had anisocytosis and 3/6 patients had relative lymphocytosis.

In the present study, 64.2% had normocytic normochromic erythrocytes. 35.8% of the patients had macrocytic anaemia and 56.3% of them had relative lymphocytosis.

Cellularity of bone marrow in aplastic anaemia[18] is very much reduced. It may be hypocellular or acellular. Lymphocytes and plasma cells are prominent. Daniel NM in their analysis of 50 cases reported 74% of patients with hypocellular marrow, 16% of patients with normocellular marrow which later became hypocellular and 10% with acellular marrow.

In the present study, bone marrow was mostly hypocellular and the aspirate was composed of fat cells in all the patients. There was a relative increase in plasma cells and lymphocytes. Bone marrow trephine biopsy revealed replacement of marrow by fat cells.

Hypersplenism

Hypersplenism is known to cause pancytopenia by sequestration of blood cells. In a study of 195 patients, Retief HP[8] found hypersplenism to be the cause of pancytopenia in 7.7% of the patients. Kumar et al. reported on incidence of hypersplenism in 19/166 cases in which ages ranged from 14–49 years. There was a male preponderance with the male to female ratio being 2:1.

In the study by Kumar et al. the Hb% ranged from 3.5–8.6 gm%. The TLC ranged from 1100–3600 cells/mm^3.

In the present study, the Hb% ranged from 3.8–10 g%, TLC ranged from 1700-3800 cells/mm^3.

Most of the patients (60%) had normocytic normochromic anaemia. 40% of them had microcytic hypochromic anaemia. In a study by Osama et al.[14] macrocytosis was seen in 63.1% cases and microcytosis in 36.8% cases. Bone marrow 66.7% had hypercellular marrow while the rest had normocellular.

Leukaemia associated with pancytopenia:

In a study by Jha et al., acute leukaemia alone constituted 90.62% of all the hematological malignancies. It accounted for 19.59% of total cases of pancytopenia. The age ranged from 2–75 years with a male to female ratio of 1.9:1. Khodke et al. and Tilak et al. reported one case of AML causing pancytopenia.

In the present study, majority (66.7%) of the pancytopenia cases were due to acute leukaemia. The ages varied from 21–40 years. There was a male preponderance with male to female ratio being 2:1.

In the study by Tilak Jain et al. one case of acute myeloid leukaemia with anisocytosis, circulating erythroblasts and immature cells was reported. Kishore Khodke et al. found one case of acute myeloid leukaemia with immature cells in the peripheral blood.

In the present study, all the patients had normocytic normochromic anaemia. Leukocyte count was reduced and circulating immature cells were seen. Platelet count was also reduced.

Myelodysplastic syndrome (MDS):

Pancytopenia is known to occur in MDS. It is the least common finding encountered in patients with MDS as compared to mono and bicytopenia.

In a study of 816 patients with MDS by Greenberg et al.[19] pancytopenia was found in 15% of the patients.

In a study of 118 patients with MDS by Juneja SK et al.[20] the age ranged from 48–95 years. In a study of 31 patients by Kini J et al.[21] the patients were in the age group of 4–7 years.

In the present study, one case presented with pancytopenia in a female patient aged 55 years.

Dengue fever

Naeem Khan et al studied 30 cases of pancytopenia and found 1 case of dengue fever. In the present study, 2 cases in 18 years (male) and 22 years (female) with dengue fever presented with pancytopenia. The total leukocyte count was 800 cells/cumm and platelet count was 28,000 cells/cumm. Bone marrow was hypercellular showing erythroid hyperplasia with a reversal of M:E ratio.

Hemolytic anaemia

Fazlur Rahim et al.[22] in their study found three cases of pancytopenia with hemolytic anaemia. Osama et al. in their study found two cases of pancytopenia with hemolytic anaemia.

In the present study, one patient of hemolytic anaemia presented with pancytopenia. Peripheral blood showed features of hemolysis. Erythrocytes showed a moderate degree of anisopoikilocytosis and an increase in number of polychromatophilic RBCs.

Reticulocyte count was markedly increased. Bone marrow was hypercellular with a reversal of M:E ratio. Erythroid hyperplasia with normoblastic maturation was seen. Megakaryocytic hyperplasia was noted in both the cases.

CONCLUSION

Megaloblastic anaemia was the commonest cause of pancytopenia in the present study. Most other studies have reported aplastic anaemia as the commonest cause. This seems to reflect the higher prevalence of nutritional anaemia, at the same time remembering the fact non nutrional causes contribute to megaloblastic change as in MDS or hemolytic anaemia. in the Indian subjects. The haematological parameters and bone marrow morphological features in patients with megaloblastic anaemia, aplastic anaemia, and malignant diseases including MDS in the present study were comparable to the findings by other authors. Uncommon etiological factors like dengue fever and hemolytic anaemia were identified in this study. A comprehensive clinical, haematological and bone marrow study of patients with pancytopenia usually helps in identification of the underlying cause. However, in view of a wide array of etiological factors, pancytopenia continues to be a challenge for hematologists. Therapeutic option for pancytopenia are drugs to stimulate blood cell production, blood transfusions, and antibiotics to treat an infection.

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FISH BONE AS A CAUSE OF RETROPHARYNGEAL ABSCESS

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Abstract

In this work, we describe the case of a 68-year-old female patient with an injury to the back wall of the pharynx by a foreign body and its atypical placement in the retropharyngeal space, causing a retropharyngeal abscess. The foreign body was extracted during a transorally direct pharyngolaryngoscopy under general anesthesia. The symptomatology, diagnosis, and therapy of retropharyngeal abscess are the topics of discussion. We emphasize the necessity of timely and thorough localization of the foreign body and its extraction.

Key words: Retropharyngeal abscess, Foreign body, Lymphadenitis, Odynophagia

INTRODUCTION

The retropharyngeal space is located between the back wall of the pharynx, the front wall of the cervical spine, and extends from the base of the skull to the mediastinum. Due to its anatomical relationship to many structures, it can cause the infection to spread to the deep cervical and interthoracic spaces. The most common pathogens are Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pneumoniae, and anaerobes. The disability is mostly paramedian and unilateral (1). The result of the inflammatory process is a retropharyngeal abscess. This is a deep throat infection that typically occurs in children between the ages of 2. and 4. year of life, most often on the basis of an abscessing lymphadenitis and after a previous infection of the upper respiratory tract. The retropharyngeal nodes often swell to the point of abscessing and can be seen as a paramedian bulge on the back wall of the pharynx /see fig.1/. Its symptoms are very similar to epiglottitis. The symptoms are dominated by fever, odynophagia, stridor, and torticollis (2). Other symptoms are headache, shortness of breath, loss of appetite, otalgia, adenopathy. A child often comes with a picture of sepsis of unclear etiology, or with symptoms of toxic laryngitis (2).

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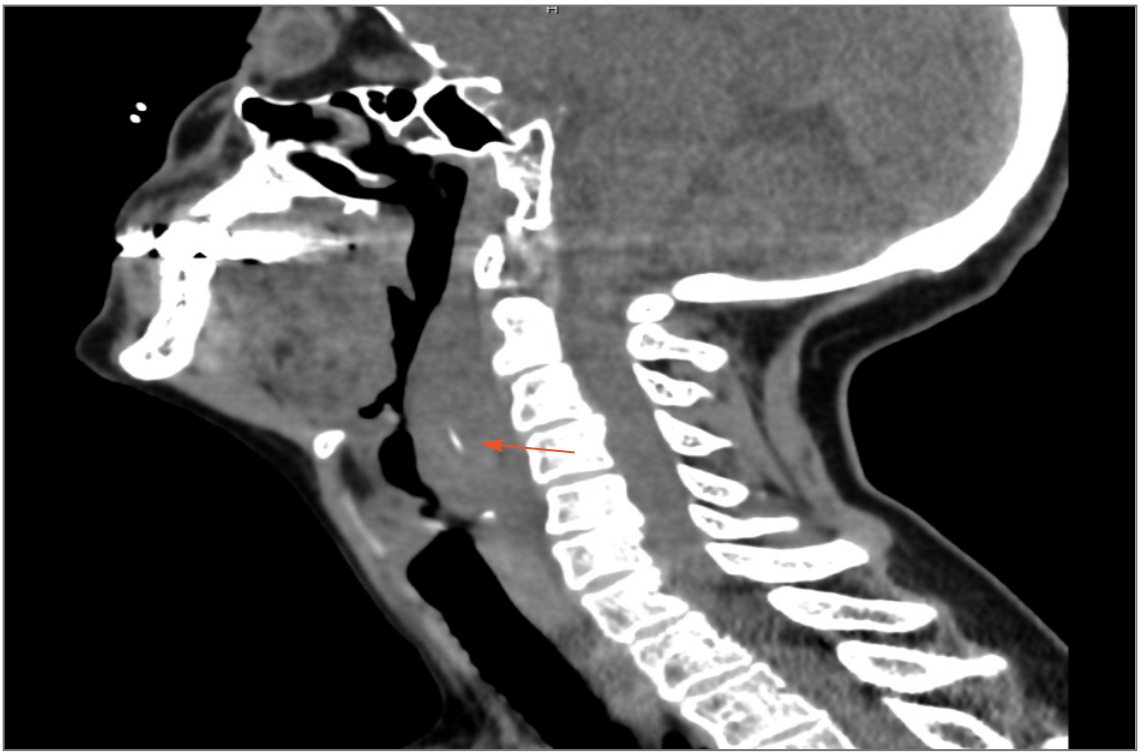

Fig. 1 CT scan of sagittal section: prevertebral soft tissue swelling with a fish bone – arrow

The incidence of retropharyngeal abscess in the adult population is lower compared to children. A foreign body can also be the cause of a retropharyngeal abscess. Foreign bodies are inorganic (needles, pins, parts of toys, dentures, etc.) or organic (fish bones, vegetables and other parts of food, etc.) (3). After ingestion, the fish bone usually gets stuck in the tissue of the tonsils, root of the tongue, valleculae, or in the side wall of the pharynx, where it subsequently causes sensations of a foreign body, pricking, scratching in the throat, or localized pain. If it is recognized late, it causes a chronic inflammation and rarely causes a deep throat infection (1). The danger of a retropharyngeal abscess lies in the obstruction of the airways, in the spread of the infection to the mediastinum with the development of mediastinitis, and a septic state. Other possible complications are aspiration pneumonia, epiglottitis, meningitis, necrotizing fasciitis, pericarditis, pyopneumothorax, and others (4). The diagnostic standard in a patient with a suspected retropharyngeal abscess is history, physical examination, and imaging methods. An ultrasound of the neck, or a CT or MRI examination is used. CT and MRI are able to accurately localize the abscess, including potential complications such as venous thrombosis (5). The basis of the treatment is surgical drainage, administration of broad-spectrum antibiotics, analgesics, antipyretics, and monitoring of airway patency or securing it by orotracheal intubation/tracheostomy (6).

CASE HISTORY

In our work, we describe the case of a 68-year-old female patient who had a history of swallowing a fish bone during the Christmas period and, due to a persistent scratchy feeling in her throat and drooling, consulted an otorhinolaryngologist, who did not find a foreign

body during the examination. Her difficulties subsided over time, and after about a month she consulted a general practitioner due to difficulty in swallowing and pain in her throat. She was taking Klacid 500 every 24 hours, despite this, her clinical condition worsened. She was subsequently treated by an outpatient otorhinolaryngologist, who suspected a retropharyngeal abscess. He sent the patient to a local hospital, where Clindamycin, Dexamethasone + Dithiaden was administered intravenously, and she was immediately transferred to our clinic. Based on the CT examination, we found an atypically located retropharyngeal abscess, practically in the middle plane, starting from the level of the tongue, spreading caudally to the left, skeletotopically to the level of C3-4, the vertebrae were without visible lesions. Under general anesthesia, we performed a direct pharyngolaryngoscopy with confirmation of an inflammatory focus. We identified a palpable soft arching approximately at the level of the upper third of the flap in the midline, after pressing, purulent contents spontaneously flowed out, from which we took a swab for bacteriological examination. We performed a wide incision and drainage of the abscess. Due to the risk of a possible postoperative swelling and suffocation with the impossibility of per os intake, we subsequently performed a tracheostomy and introduced a nasogastric tube. The operation went without complications. Postoperatively, due to subjective persistent problems, we completed a control CT examination of the neck and chest /see fig.2/, where a residual abscess collection of the retropharynx was confirmed in the form of a streak with a discrete suspicious foreign body. A small seepage was detected around the tracheostomy cannula and the fat of the upper mediastinum, where suspicious inflammatory changes were identified without confirmation of an abscess.

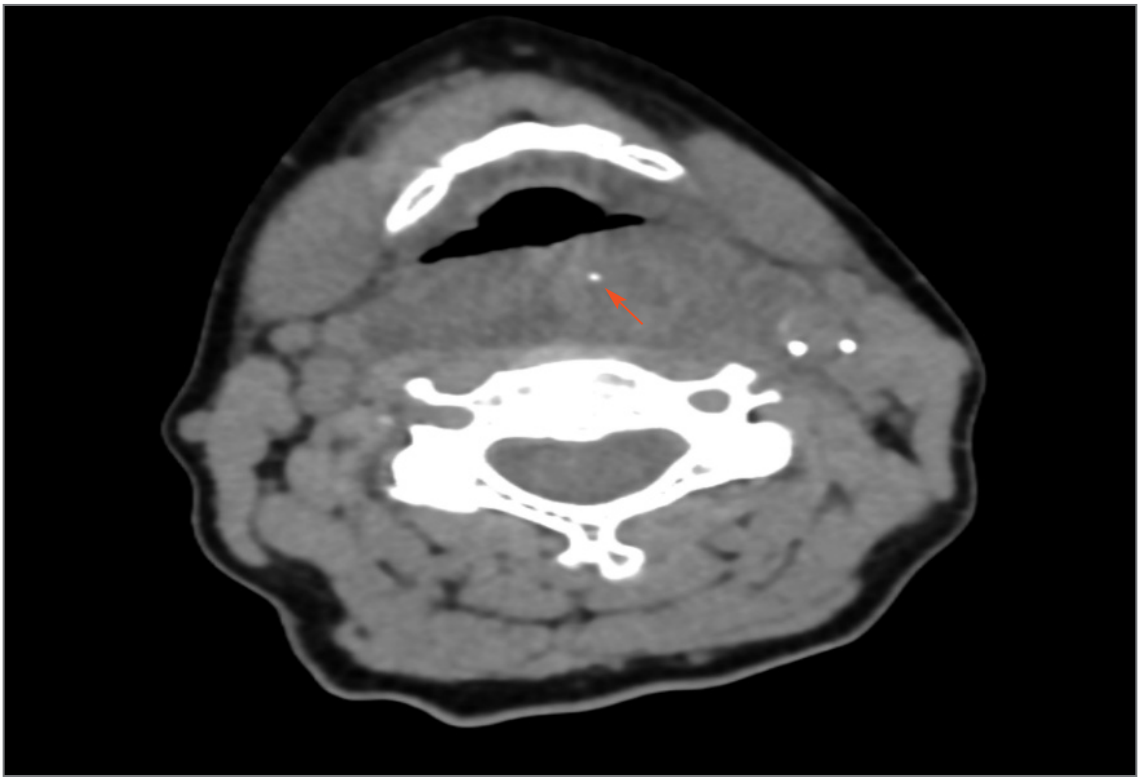

Fig. 2 CT scan, axial section, foreign body – fish bone 15 mm – arrow

During the revision operation, we removed the fibrin coating in the place of the original incision with a Kleinsasser laryngoscope. We dilated the incision and revised the soft tissues up to the prevertebral fascia – in depth we identified the foreign body described according to the CT scan (approx. 15 mm long thin fish bone), which we removed /see fig. 3/. We did not find purulent content, we only aspirated a minimal amount of calcified content. Initially, in the laboratory parameters, significant leukocytosis and elevation of C-reactive protein. Doses of antibiotics (Clindamycin 600 mg IV every 8 hours and Metronidazole 500 mg IV every 12 hours) were not changed during the hospitalization, they corresponded to the culture findings. Postoperatively, the laboratory parameters are adjusted, the subjective problems subsided. On the 5th postoperative day, we extracted the nasogastric tube. The process of realimentation per os was without difficulties. We decannulated the patient on the 6th postoperative day – without breathing difficulties. In a stabilized condition, we discharged the patient to outpatient care on the 8th postoperative day.

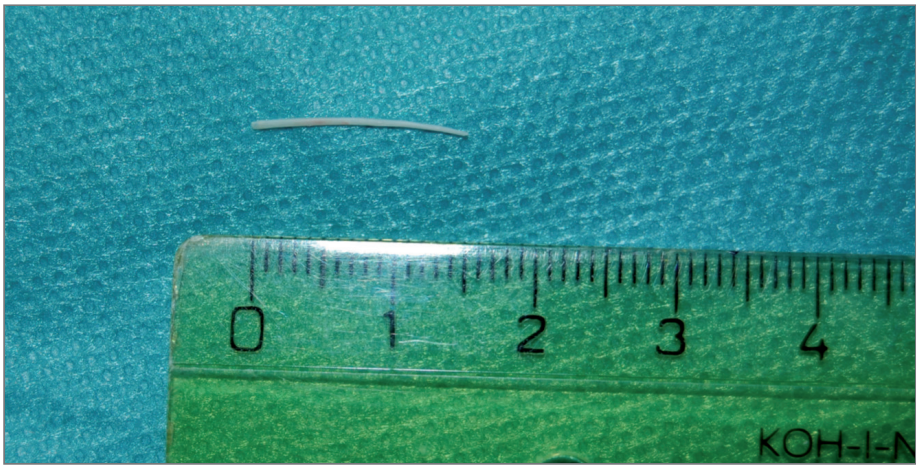

Fig. 3 Foreign body – fish bone 15 mm

DISCUSSION

As otorhinolaryngologists, we come into contact with foreign bodies most often after their ingestion or traumatic penetration. After ingestion, the fish bone usually gets stuck in the tissue of the tonsils, root of the tongue, valleculae, or in the side wall of the pharynx, where it subsequently causes sensations of a foreign body, pricking, scratching in the throat, or localized pain (1). In the case of our patient, a foreign body stuck atypically in the area of the retropharynx and caused an abscess collection. The diagnosis of foreign bodies is important, so do not underestimate the situation and perform an imaging examination, or repeat it in case of an ambiguous result. It will reveal the exact location and help assess which anatomical structures are affected. CT examination is better available in our country, but NMR can also be used. Foreign bodies are often metallic, plastic, glass and are easily detected by X-ray (5). A foreign body made of wooden material can sometimes escape our attention because it is of lower density and because gas bubbles form around it (6). In open injuries, NMR examination is controversial because of the possible occurrence of metallic foreign bodies. In the mentioned literature, in some cases, a foreign bodies – metal fragments smaller than 1x1 mm – were not shown on the CT examination (7). Some authors also use angiographic methods when there is no clarity of vascular involvement and the risk of major bleeding. In the patient we presented, the diagnosis was established on the basis of a clinical suspicion (persistent lymphadenopathy of the neck, dysphagia, odynophagia),

physical examination, and CT of the neck and chest with contrast. The foreign body must always be removed completely, including its particles. There may be chronic inflammation, recurrences with fistulation and suppuration, or the formation of inflammatory granulomas (5,8). When extracting foreign bodies, the correct operative approach is important. We consider the size, extent, location, type of material of the foreign body, and possible complications. We choose an approach that can remove a complex foreign body and we also consider mini-invasiveness (9). However, foreign bodies made of organic material are often fragile and their complete extraction is more difficult compared to e.g. with metal material (6,12). Operations in the stage of acute inflammatory manifestations should be performed under the cover of antibiotics (10). If an abscess is not formed and there are signs of cellulitis, conservative treatment is sufficient. We choose antibiotics with sensitivity to gram-positive bacteria and anaerobes, i.e. clindamycin, cephalosporins II.-III. generation or potentized aminopenicillins. (1.11) When the surgical intervention is delayed, there is a risk of infection penetrating from the retropharyngeal space into other spaces (“danger space”, parapharynx, mediastinum) with the risk of fatal complications (3). In the case presented by us, the initial foreign body was not expected due to the atypical localization at the site of the abscess collection, and it was not possible to immediately identify the fish bone. On the basis of persistent clinical problems and elevated inflammatory parameters in the laboratory, we added control CT scans of the neck and chest. A residual abscess collection of the retropharynx with a foreign body and subsequent revision surgery is described here. In our patient, the foreign body was accessible due to its atypical placement, with good visualization and manipulation, sufficient control of possible bleeding, and inspection after the removal of the body. As part of the surgical treatment, we primarily chose a transoral approach for the diagnosed abscess, as in tonsillectomy, or using instrumentation for mini-invasive surgery of head and neck tumors. Some authors choose an external approach as safer and more beneficial. Dissection begins at the front edge of the rocker into the parapharyngeal space and can continue directly to the vertebral bodies into the retropharyngeal or prevertebral space (1).

According to the Czech authors, the transoral approach can be used in the absence of signs of parapharyngeal spread. The incision should be wide enough to drain and below the apex of the arch to avoid creating a pocket with the possibility of secretion retention (1). Surgical drainage of an abscess through an external approach involves a wide opening of the fascial spaces of the abscess cavity, identification of the major jugular vessels and their control, drainage of all separated abscess pockets, irrigation of the wound, and insertion of wide tubular drains (1). We always take material for bacteriological examination. Depending on the extent of the finding, they eventually introduce a nasogastric tube to ensure postoperative nutrition (13). Therefore, the external approach should be used in case of extensive infections with a spread to the parapharyngeal space or mediastinum or an oppression of the respiratory tract. Secure the elector's airway in case of shortness of breath. Exceptionally, only intravenous antibiotics are used in the treatment of retropharyngeal abscesses and are not identified with this procedure (1). In the Austrian literature, transoral is chosen as the primary approach. They also recommend an external approach when the abscess spreads to other spaces ("danger space", parapharynx, mediastinum) or in case of complications. For larger abscesses, food intake must be ensured using a nasogastric tube. Good visualization is necessary during the procedure and also hemostasis after the extraction (11). At our workplace, according to the clinical findings, we also indicated the securing of the airways using a tracheostomy. The mentioned authors consider this procedure in the case of shortness of breath. Bleeding from large vessels, or the risk of fatal complications, is also a feared complication (14). Postoperatively, inflammatory complications may occur, which may be local or general. Among the most feared are mediastinitis or the development of a septic condition. Other possible complications are aspiration pneumonia, epiglottitis, meningitis, necrotizing fasciitis, pericarditis, pyopneumothorax, and others (4,13). Therefore, it is important to consult a thoracic surgeon. Due to the absence of an abscess in the chest cavity, our patient's case did not require a collar mediastinotomy.

CONCLUSION

Traumatic penetration of a foreign body into the retropharyngeal space is rare for otorhinolaryngologists. Diagnostics using imaging examinations to determine the size and location of the foreign body, as well as possible inflammatory complications in the area of the neck and mediastinum, as in our case report, is important. We always choose the surgical approach to remove the foreign body in such a way that a complete extraction is possible. Subsequently, we consider the mini-invasiveness of the surgical procedure while keeping in mind the possibility of bleeding, so we choose an approach with good visualization. We try to prevent damage to important anatomical structures and prevent postoperative complications. It is necessary to think about this diagnosis especially in the framework of differential diagnosis. In addition to the occurrence of a foreign body in this area, other causes of diseases such as injuries, developmental or tumor diseases should be considered (2).

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