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A multifactorial complex of aetiology pathogenic factors of schizophrenia

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ABSTRACT

Schizophrenia aetiology pathogenic factors involves a complex of mechanisms which have not been fully clarified yet. The data in the literature stressing out the action of factors from the genetic, neurobiological, neuro-biochemical and psycho-social spectrum. The genetic determinism of the disorder is modelled by the interaction with the environment factors, in the context of vulnerability – stress diathesis. Cerebral structural abnormalities represent the best-defined support of the aetiology pathogenic neurobiological model of schizophrenia, neuroimaging studies objectivising changes at the level of the cerebral areas involved in the debut and evolution of disorder and engendering two important aetiology pathogenic hypotheses, the neurodegenerative one and the neurodevelopment one, as well. Neurobiochemical vulnerability is supported by the emphasising of alterations in the neurotransmitters balance, based on these data being built the following hypotheses: dopaminergic, serotoninergic, noradrenergic, glutamatergic respectively. Psycho-social factors involved in the aetiology pathogenic factors of schizophrenia and psychotic disorders are represented by vulnerabilities at individual, social micro-, macro group respectively level, being emphasised in this category the lacks in the socio-economic support, discrimination, urban life style, the action of stressful factors, emotional hyper expressivity or dysfunctionalities at the family level.

The specificity and complexity of schizophrenia thus represents a challenge for clinicians and researchers, both through aetiology pathogenic mechanisms, and the evolution of the condition, and the effects it has upon the individual and the community he/she is part of it.

Key words: genetics, neurobiology, neurotransmitters, psycho-social factors, schizophrenia.

Introduction

Schizophrenia is one of the major psychiatric disorders, which, apart from the special clinical aspects, also implies a whole series of economic and social costs, direct and indirect, costs required both by the disorder specificity, and the fact that it affects approximatively 1% of the earth's population [24], at the same time also representing a severe public health problem through its disability effects. Talking about schizophrenia, we must have in view that it is a condition that does not have a geographic, cultural or social determinism, showing the same symptomatology in any environment, and this, corroborated with its deep medical and economic implications, entails it being considered a priority in psychiatry and in the public health worldwide. [34]

Schizophrenia aetiology pathogen involved factors

Vulnerability – stress diathesis represents one of the attempts to phrase an answer, as complete as possible, to the necessity of explaining the aetiology pathogenic mechanisms of schizophrenia, the combination between the biological factors and the psychosocial context being seen as a main cause for the disorder onset, dependent both upon the intensity of the stress factor, and upon the potential of biological vulnerability of the affected individual.

Biological Vulnerability

Most of the theories which tried to explain the schizophrenia aetiology pathogenic factors have been centred upon the biological vulnerability, a complex mechanism which involves genetic, neurobiological, neuro-biochemical and immunological determinism. Thus, they tried to provide an explicative model based on evidences and which might represent the background of therapeutic strategies and of interventions for the purpose of preventing the condition appearance and development.

Genetic Determinism

The data provided by researches in the genetics spectrum have emphasised the role that these factors have in the schizophrenia aetiology pathology, especially in the context of vulnerability – stress diathesis, in which genetic influence is modelled by the interaction with the environmental factors.

Thus, in the case of studies upon monozygotic twins it could be noticed a disorder accord of up to 80%, as compared to a rate of only up to 30% for dizygotic twins [27].

Moreover, studies performed in the case of subjects of adoption processes have proved the fact that the risk of schizophrenia onset is identical both in the biological family, and in the adaptive one, with an enhancement for the children whose biological parents did not suffer from schizophrenia, but who subsequently grew up within a family in which at least one of the adoptive parents was set this diagnosis [21].

Up to presently, the attempts to identify genes running for the risk of schizophrenia development did not have probative results, being established correlations with those involved in the Catechol-O-methyltransferase (COMT) mechanisms [33] or in the activity of dopaminergic receptors [17], as well as at the chromosomal level, especially to the chromosomes 22q, 8p respectively [25, 28].

Neurobiological Factors

Within this category of factors, there are mainly intricate the neurodevelopment abnormalities which were emphasised both with people with the set diagnosis of schizophrenia, and with members of their families, therefore having a direct correlation with the previously set out genetic determinism, even if these neurostructural abnormalities are not directly associated with cerebral lesions.

In this context, the discussion was around about a neurobiological model of schizophrenia based on structural abnormalities and cortico-subcortical dysconnectivity [3], the neuroimaging studies emphasising with preponderance the presence of ventriculomegaly, of atrophy of the temporal lobes and of the frontal lobe, as well as lesions of the limbic and paralimbic system, in this category falling the following structures: hippocampus, para-hippocampus, orbital cortex, cingulate gyrus and cerebral amygdala [31].

Based on the nuclear magnetic resonance imaging studies (MRI), there were emphasised as primordial structural alterations in variations comprised between 50-90% respectively in cavum septi pellucidi, lateral ventricles, limbic system, third ventricle, basal ganglions, upper temporal gyrus, callous body, temporal, frontal and parietal lobes [26].

These cerebral structure abnormalities associate the functionality deficits which represent the main syndrome feature of schizophrenia. Thus, deficiencies in the field of executive functions are engendered by structural alterations of the prefrontal cortex, responsible for the cognitive processes which represent the background of these executive functions, in the case of schizophrenia being affected the whole spectrum of constitutive elements: volitional and conscious processes, the planning and abstracting capacity, thinking flexibility and adaptiveness, cognitive abilities related to the initiation, sustain and finishing an action [15].

Much more important are the cognitive

deficits, the central element of the symptomatology which is specific to schizophrenia, with direct effect upon the functional capacities of the individual affected by the disease and of the quality of life [14].

Deficit of the cognition is fully acknowledged in the case of schizophrenia, being noticed its deficiencies even from ahead of condition prodrome, the cognitive impairment being noticed right from the childhood of individuals with this diagnosis [9]. The cognitive deficit is amplified during the chronic evolution being one of the main generators of the burden of the disease by affectation of the individual and social functionality, with direct consequences upon the quality of life and especially upon the functional independence [19].

A significant characteristic of schizophrenia, cognitive deficit is manifested in all areas of this psychological process, being affected the ability to assimilate, the speed of information processing, especially through attention deficits and memory, in the case of the latter especially its central component, but also the visual-spatial and verbal ones [5]. Functional neuroimaging studies have highlighted that structural anomalies at the prefrontal dorsolateral cortex are responsible for this central memory component deficiency, including disconnections and alterations in neuronal neurotransmission [22].

An important element of the cognitive spectrum, the social cognition, the process involved in the way the individual perceives and thinks about himself and others, interprets social situations and reacts to them, also presents a marked deficit for people with schizophrenia. These deficits of social cognition are also encountered in the perception and correct interpretation of visual contact in spatial language, by erroneous interpretation of the emotions of others and alienated inferences, being affected the decisional capacity in social life and generating delusional ideation and anxiety [2].

Individuals with schizophrenia have also been exposed to language deficiency, particularly in terms of expressive and responsive language, visual perceptual abilities and fine motor skills [5].

Thus, discussing about the neurobiological vulnerability and its consequences should also be taken into account the microstructural abnormalities materialized by quantitative alterations of the grey matter and the quality of the white substance, the increase of the neuronal density while reducing the dendritic trees and the cortical volume [4].

In this context it can be considered that all biological structural anomalies are some with an evolutionary trajectory which, on a vulnerable genetic background and under the subsequent action of the environmental factors, lead both to the occurrence of the disease and to the amplification of its clinical manifestations.

Thus, two of the most important etiopathogenetic hypotheses of schizophrenia, neurodegenerative and neurodevelopmental can be confirmed, the data provided by the neuroimaging studies providing evidence in the support of both but at the same time contributing to a comprehensive vision expressed by the concept of neuroprogression, according to which in schizophrenia, the structural-cerebral changes are present before the onset, but with the first episode they are amplified, and later, along with the evolution of the disease, they will be accompanied by new ones [4].

Neurobiochemical vulnerability

The neuro-biochemical aetiology pathology mechanisms of schizophrenia refer to changes in cerebral neurotransmitters balance, deciphering these mechanisms underlying the development of psychopharmacological therapeutic strategies based on antipsychotics. Research over time has been able to outline aetiology pathogenic patterns of schizophrenia for the imbalances of each neurotransmitter, models that do not yet constitute a unitary picture and should be considered based on both vulnerable genetic and biological factors and the clinical aspects of disease.

The dopaminergic hypothesis was first developed chronologically and explained the antipsychotic effect of the first neuroleptic substances and revealed correlations between the onset of schizophrenia and the hyperactivity of some brain structures, especially in the limbic system, the excess of mesolimbic dopamine release leading to positive psychotic symptomatology, as well as a dopamine deficiency especially in mesocortex, resulting directly in negative symptomatology and cognitive deficit [32]. Abnormalities of the dopamine release

are manifested at presynaptic level, dopaminergic firing affecting neuronal circuits and leading to psychotic symptoms through aberrant dopaminergic transmission in the striatum and increased D2 receptor binding potential in the frontal cortex [1].

At the prefrontal cortex level, functional disruption between D1 / D2 receptors is responsible for producing positive and negative symptoms, while the latter have causality in the prefrontal cortex - limbic system (especially cerebral amygdala) [16]. Thus, based on neurofunctional studies, primary hypo-frontally phenomena generated by the inhibition of dopamine release in the dorso-medial frontal cortex and secondary to the striatum have been described, where the blockade of D2 receptors results in an imbalance in the dopamine - Gamma Amino-Butyric Acid (GABA) ratio and to a neurotoxic effect produced by hyper-glutamate activity. This hypothesis, based on the evaluation of the effects of dopamine agonists or antagonists substances, has also demonstrated marked by the limits on the non-therapeutic response to these substances in more than 30% of schizophrenic patients, the limited efficacy of these drugs against positive symptoms and the modulator effect on other receptor types [8].

The serotoninergic hypothesis of schizophrenia was also based on the study of the effects of antipsychotics on both serotonergic transmission pathways and cerebral serotonin receptors, with not only therapeutically positive results, but also the reduced side effects induced by these substances [23].

The theory that emphasized the role played by norepinephrine in the aetiology pathogenicity of schizophrenia was based on the mechanisms of heterogeneity between the dopaminergic system and the cerebral noradrenergic system, the modulation in activity and the number of noradrenergic receptors being directly associated with the occurrence and degree of severity of the negative symptomatology, as well as with psychomotor excitation syndrome [6].

Greater importance was given to the glutamatergic hypothesis, a theory that was constructed from the observation of a quantitative and qualitative-functional glutamate-N-methyl-D-aspartate (NMDA) receptors in the prefrontal, temporal cortex, mesolimbic structures and hippocampus, in persons diagnosed with schizophrenia, a deficit which was considered responsible for the occurrence of psychotic symptoms through dopaminergic hyperactivity [30].

Psychosocial vulnerability

Schizophrenia is one of the eloquent examples of mental health disorder in which the bio-psycho-social holistic perspective must be considered primarily when discussing the aetiology pathogenic mechanisms that underlie the onset and evolution of the disease.

Theories that conceptualized models based on multiple, biological and environmental influences have highlighted the important role that psycho-social factors acting at all levels, micro- (individuals and families) or macro- (social group, community, society) they have it in the determinism of the occurrence of the disease, both as potential triggers, and as emphasizers of other elements complicated in the aetiology of schizophrenia. Moreover, the importance of the effects of this category of factors on the evolution of the disease, the compliance and adherence to the therapeutic management program, or the process of social recovery and reintegration was emphasized.

Initial researches focused on aspects of social origin as a potential disruptive factor of mental health and the generating of psychotic symptoms, thus elaborating the socio-genetic hypothesis of schizophrenia, from the perspective of which belonging to a disadvantaged social group is both a risk factor for the occurrence of the disease, and one that leads to an unfavourable, chronic evolution, with multiple episodes and poor therapeutic response [11].

Subsequent complex epidemiological studies have partially invalidated this hypothesis, showing that schizophrenia affects individuals from diverse social groups with diverse socio-economic statuses and, moreover, were underlined high rates of disease incidence in socially and economically developed countries, which can provide social support for people in disadvantaged categories [18]. However, it was validated the observation that the urban environment, especially the over-crowded areas, is a risk factor for the disease, together with the marital status of the affected individuals, unmarried persons present significantly higher

risks for the disease than those involved in marital relationships [12].

The hypothesis of psychosocial vulnerability has given a particular position to the family, the research carried out in this regard demonstrating the existence of a functional deficiency at this level, a deficiency manifested in the main areas of psychosocial support that the microfamily group has to provide: good communication, affectivity, support in problem solving, fulfilment of the social roles associated with each member, behavioural control [13]. Familial dysfunctions lead to the amplification of cognitive impairment in people diagnosed with schizophrenia, particularly in the social field of cognition, which can be translated into psychologically inappropriate social perception of the living environment, social situations, stimuli and interactions, leading to unfavourable disease progression, with multiple episodes and with a high degree of severity and serious impairment of quality of life.

Against this background, the concept of social defeat, which presents a cortical reflection in the hyperactivity of the mesolimbic system at the D2 striatal dopaminergic receptors, is a major risk factor for both the onset of the disease and its subsequent development. Social defeat was identified especially among disadvantaged social categories (migrants, people with low socio-economic status), the perception of negative social experiences being correlated with the emergence of psychotic symptoms [29].

Through direct effects on cognitive abilities, especially memory enhancement, psycho-trauma events are involved in both onset [20] and in the subsequent evolution of schizophrenia, being previously evidenced to a recurrence of over half of people with schizophrenia [7].

The kindling phenomenon was thus addressed (abnormal neuronal response to a repetitive stimulus) manifested in the limbic system, especially in cerebral amygdala, which affects the balance of neurotransmitter systems, especially dopaminergic and glutamatergic systems, resulted in a cognitive deficit and the risk of psychotic symptoms [10].

Conclusions

The specificity and complexity of schizophrenia is thus a challenge for clinicians and researchers, the symptomatology that combines both functional and cognitive deficits, affecting the quality of life of people with this diagnosis, as well as their families or the social group which they are part of.

Severe, chronic and invalid maladies, schizophrenia is thus at the end of complex and yet incompletely identified aetiology pathogenic pathways. Aetiology pathogenic patterns of disease include directions that go from genetic baggage to the role of stress or environmental factors, with variants that translate into neurotransmitter dynamics or changes in neuronal circuits and which summed up, lead to the onset and evolution of the disease with a full spectrum of effects on the affected individual, and of the community he belongs to.

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Нови биомаркери в ръководствата за диагностика на железен дефицит и анемия при някои хронични възпалителни заболявания и клинични състояния

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New Biomarkers in the Guidelines on the Diagnosis of Iron Deficiency Anemia in Several Chronic Inflammatory Diseases and Medical Conditions

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РЕЗЮМЕ:

Железният дефицит и анемия са познати клинични състояния, често свързани с някои хронични заболявания и възпаление, и може да доведат до обостряне, влошаване на клиничното състояние и изхода от заболяването. Професионалните медицински сдружения и асоциации от различни специалности са изработили свои ръководни указания за диагностицирането на железен дефицит и анемия при тези състояния. В статията се разглеждат някои нови биомаркери на тъканен железен дефицит и хипохромия, чиято диагностична роля се споменава в ръководствата, като информативни за желязодефицитна еритропоеза и в условия на възпаление.

Ключови думи: биомаркери, хипохромия, дефицит на желязо, анемия, диагноза, ръководства.

ABSTRACT

Iron deficiency and anemia are common medical conditions that are frequently concomitant with several chronic diseases and inflammatory states, and may cause clinical deterioration, disease exacerbation and poor outcome. Professional medical associations of different specialties have developed their own guidelines on the diagnosis of iron deficiency and anemiain these medical conditions. This article reviews some new biomarkers of tissue iron deficiency and hypochromia, which diagnostic role is mentioned in the guidelines, as informative of iron-deficient erythropoiesisseven in the presence of inflammation.

Key words: biomarkers – hypochromia – iron deficiency – anemia – diagnosis – guidelines.

Introduction

Iron deficiency (ID) and iron deficiency anemia (IDA) are common medical conditions in everyday clinical practice [11]. They are well characterized and easily diagnosed based on a few established biochemical and hematological markers [12]. Severalmedical conditions and chronic diseasesare frequently associated with ID with or without anemia as common complication- inflammatory bowel diseases (IBD), chronic kidney diseases (CKD), patients on hemodialysis (HD), chronic heart failure (CHF), and cancers [11,21,27]. Iron deficiency may be either absolute with depleted iron stores or functional ID when iron is sequestrated in the macrophages of the reticuloendothelial system. Functional ID is a characteristic of anemia of chronic disease (ACD) or may present the insufficient mobilization of iron from stores in the presence of increased demands, e.g. after treatment with erythropoiesis-stimulating agents (ESAs) [11, 47]. In chronic inflammatory states ID and anemia are frequently more complex to recognize and diagnose by conventional biochemical and hematological laboratory parameters, and often neglected, but their impact may lead to severe condition andexacerbations, poor quality of life and disease outcome.Some new biomarkers of tissue ID and hypochromia appear useful for diagnosis as informative of bone marrow iron demand and iron-restricted erythropoiesis due to absolute or functional ID.

Conventional diagnostic tests in ID and IDA

Serum iron and transferrin saturation (TAST) are commonly used laboratory tests fordetecting ID, because they are available and inexpensive.However, food intake, rapid iron turn-over andcircadian changes in iron concentrationcause fluctuations of TSAT [15]. Transferrin synthesis and clearance may vary in conditions like protein deficiency, chronic diseases and infections, liver diseases, nephritic syndrome and influence TSAT. Both decrease in serum iron and concentration of transferrin, which is a negative acute-phase protein, may change TSAT in inflammation [24].These factors have serious impact on reliability of the mentioned parameters and reduce the specificity of TSAT. There is also marked overlap in the values of these indices between normal and iron-deficient individuals.

In the absence of inflammation the measurement of serum concentration of ferritin is the most specific test that correlates with body iron stores [22]. Its main advantages are accessibility, lowcost, available standardization and high sensitivity to the variations of the iron stores. Iron deficiency is the only cause of low serum ferritin concentration, but it may be increased independently of iron status in acute and chronic inflammatory disorders, malignancies, liver, autoimmune and metabolic diseases, which poses serious diagnostic limitations [27, 49].

Iron deficiency anemia is characterized by low mean erythrocyte indices – mean cell volume (MCV) and mean cell hemoglobin (MCH), and increased red cell distribution width (RDW). Although not affected by inflammation, their change occurs late because of long life span of red blood cells (RBC) and have low specificity for ID [9, 12].

New biomarkers in the diagnostic guidelines of iron deficiencyand anemia

Professional associations of different specialties – anesthesiology, cardiology, gastroenterology, obstetrics and gynecology, hematology, nephrology, oncology, orthopedics, and pediatrics, have developed their own guidelines on the diagnosis of ID and anemia [13,35]. According to systematic reviewsserum concentration of ferritin was recommended by all guidelines for the diagnosis of ID anddefining ranges varied from 12 to 100μg/l for absolute ID and from 100 to 800μg/lfor functional ID [13,35]. TSAT was proposed as an alternative or complementary diagnostic test in nearly half of the guidelines with thresholds broadly similar for absolute and functional ID and ranged from 15 to 30% [13,35].

In recent years, diagnostic role of new biomarkers has been mentioned in the guidelines on the diagnosis of ID and anemia in different chronicinflammatory diseases and medical conditions (Table 1). Several guidelines even suggest threshold values.

Soluble transferrin receptor and sTfR-ferritin index

Soluble transferrin receptor (sTfR) in serum derives from proteolysis of the membrane transferrin receptors of erythroblasts and reticulocytes, and its concentration is proportional to the amount of cellular receptor and erythroid precursors in the bone marrow. In case of ID, transferrin receptor synthesis is increased, leading to corresponding increase in sTfR, which makes sTfR a biomarker of tissue ID and bone marrow demand for iron [17, 30, 41]. Iron-deficient erythropoiesis is the most common cause of elevated sTfR [14].The sTfR is not significantly influenced by infection or inflammation and appeared efficient for differentiating ID in ACD [37].The sTfR measurement is suggested in the diagnostic approach of ID in patients with complicated anemia, typical for concomitant inflammatory disease when ferritin concentration is increased [2, 4].The biomarker may also be useful for detecting functional ID irrespective of the status of iron stores[14, 18].It seems to improve the clinical diagnosis of IDA, especially in chronic disease or gastrointestinal neoplasms [26], but a meta-analysis identified sTfR as a fairly good test for IDA screening,and not for confirmatory purposes [23].

Soluble transferrin receptor concentrations can be raised in conditions with stimulated erythropoiesis like autoimmune or hereditary hemolytic anemia and megaloblastic anemia [2, 41]. Another limitation is lack of standardization among different available methods, and universally determined reference ranges and cutoffs, which makes the results method dependent and incomparable [17, 27, 41].

The ratio between sTfR and the logarithm of serum ferritin (sTfR/log10ferritin), named sTfR-F index, has been suggested as a biomarker that fully assesses body iron stores, because it isdirectly proportional to the measurement of the functional and storage iron compartments. It takes advantage of the relationship between two phenomena in ID, i.e., an increase in sTfR and a decrease in the ferritin concentration - two variables, which are influenced by the body iron stores and the availability of iron for erythropoiesis. Initially studied sTfR-F index provided an outstanding indicator of iron depletion and highersensitivity and specificityin comparison to the use of sTfRor ferritin alone, for the detection of ID in anemia in inflammatory conditions [37].Non-anemic ID statesare also more easily detected by sTfR-F index, when distinguishing ID and iron-deficient erythropoiesis. In borderline cases, when the results of sTfR and ferritinare ambiguous, the sTfR-F index presents as more sensitive for detecting iron-deficient states [42].The usefulness of the sTfR-F index in differentiating IDA and ACDhas subsequently been ascertained [39, 40], and although the limits are not clearly defined, a ratio more than 2 was suggestedfor absolute ID in ACD [47].Probably the main limitations for the widespread clinical use of the sTfR-F index are the same as of sTfR.

Hepcidin

Hepcidin is a peptide mainly synthesized in the liver with central role in systemic iron homeostasis. It controls both iron absorption and store release. Hepcidin has been studied in recent years as a biomarker for the diagnosis of iron disorders. It decreases in ID and increases in ACD. Its levelsin combined anemia appear to be lower than in ACD, as it is influenced more strongly by the erythropoiesis need for iron than by the inflammation [44], but its discriminatory potential for both conditions is not sufficient for a clinical decision and is used mainly in experimental settings[48]. Hepcidin testing lacks standardization and levels show circadian variations, results require interpretation in the light of clinical context with testing for inflammation and for hepatic and renal function disorders – conditions that alters hepcidin concentration significantly due to changes in its synthesis and clearance [19].

Reticulocyte hemoglobin content

Contemporary automated blood cell counters that apply flow cytometry technology report parameters that provide information about individual cell characteristics in addition to calculated mean values of the total red cell population [46].

Reticulocytes are immature erythrocytes precursors and their conversion to mature

Table 1. ***Biomarkers of hypochromia and tissue ID in guidelines on the diagnosis of ID and anemia in several chronic diseases and medical conditions.***

Professional association	Year	Disease/ Condition	Biomarker	Status	Context
Gastroenterology					
BSGE[20]	2011	Digestive diseases	sTfR, sTfR-F index	cited	Marker of ID in healthy subjects and discrimination in chronic disease.
			CHr, %HRC	cited	Early indication of FID and prediction of treatment response.
ECCO[16]	2015	IBD	sTfR, CHr, %HRC	suggested	Measurement can be considered in uncertain cases of ID, IDA.
			sTfR-F index, CHr, %HRC	suggested	To excludetrue iron deficiency (sTfR-F index) and to diagnose FID in ACD.
Portuguese Working Group on IBD [28]	2016	IBD	sTfR	recommended	Can beuseful to detect ID in patients with anaemia and TSAT< 16% andferritin (30-100mg/l)
			sTfR-F index	recommended	sTfR-F index<1is useful to exclude ID in patientswith chronic inflammation anaemias. WhenTSAT<16%and Ferr 30-100mg/l sTfR-F>2 – indicates mixed anemia (IDA + CDA), sTfR-F<1 indicates ACD (in algorithm)
			%HRC	cited	Limited usefulnessto detect IDA in IBD.
			CHr	cited	Could be a particularly useful in the evaluation of the therapy response of ID in IBD.
			Hepc	cited	Scarce literature, not standardized.
Nephrology					
BCSH [45]	2013	CKD, HD	%HRC	recommended	Identification of FID - best-established variable.
			CHr/Ret-He	recommended	CHr<29 pg predicts IRE in patients with IDA, FID and those receiving ESA therapy. Ret-He<25pg predicts FID in those receiving ESA therapy; Ret-He<30.6pg appears to be the best predictive value forresponse to i.v. iron in CKD on HD.
			sTfR	suggested	Alone or as sTfR-F index, if %HRC, CHr or Ret-He are unavailable.
			Hepc	cited	Uncertain utility as a diagnostic tool and remains a research investigation.
UK NICE [34]	2015	CKD, HD	%HRC	recommended	Diagnose ID, determine potential responsiveness to irontherapy and long-term iron requirements %HRC>6%
			CHr/RetHe	recommended	CHr<29pg (or equivalent tests), when %HRC testing is not possible
UKRR [32]	2017	CKD	%HRC, CHr/Ret-He	recommended	Evaluation of anemia - test to determine iron statusand available iron for erythropoiesis. CHr<29pg (or equivalent tests) is more sensitive in determiningiron depletion than %HRC.
Cardiology					
SEC-SEMI [29]	2017	HF	sTfR	suggested	sTfR>1.59mg/l demonstrates an increased cell iron demand.Diagnosis of ID, especially useful in doubtful cases. ID in patientswithFerr 300-800µg/l and TSAT<20% (in algorithm)
			CHr, %HRC	cited	Iron-deficient erythropoiesis - CHr<28pg; early marker of responseto the iron therapy, %Hypo–later one.
			Hepc	suggested	Hepc<40µg/ml, indicative of depleted iron deposits.
Anesthesiology					
AAGBI [33]	2017	Pre-operative anemia and ID	CHr, %HRC	suggested	Confirm ID component in thesetting of inflammation-CHr<28pg or %HRC>5%, suggests iron supplementationmay be beneficial.
			sTfR-Findex	suggested	sTfR-F index>2 indicates true IDin thesetting of
					inflammation,strong predictor of response to i.v.iron, when CHr, %HRC are not routinely available.
Oncology					
ESMO [1]	2018	CIA	CHr, %HRC	suggested	Parameters for impaired iron status - %HRC>5%, CHr<28 pg.
			sTfR	cited	Limited relevanceas an indicator of iron status.

AAGBI – Association of Anaesthetists of Great Britain and Ireland; ACD - anemia due to chronic disease; BCSH – British Committee for Standards in Haematology; BSGE – British Society of Gastroenterology; CHr - content of reticulocyte hemoglobin; CIA - chemotherapy induced anemia; CKD - chronic kidney disease;ECCO – European Crohn's and Colitis Organisation;ESMO – European Society of Medical Oncology; FID - functionaliron deficiency;HD - hemodialysis; Hepc – hepcidin; HF – heart failure; %HRC - hypochromic red cell; IBD - inflammatory bowel disease; ID - iron deficiency; IDA - irondeficiencyanemia; IRE – iron restricted erythropoiesis; Ret-He - reticulocytes hemoglobin equivalent; SEC- SEMI– Spanish Society of Cardiology andSpanish Society of Internal Medicine; sTfR - soluble transferrin receptor; sTfR-F index - soluble transferrin receptor/log ferritin index; TSAT - transferrin saturation; UK NICE– National Institute for Health and Care Excellence; UKRR – UK Renal Association and Registry.

erythrocytes takes 3 to 4 days, first in the bone marrow and in the last 1-2 days - in the circulation. Circulating reticulocytes do not synthesize hemoglobin, unlike reticulocytes in the bone marrow. Because of their short life span,the measurement of their hemoglobin content (CHr) reflects the presence of functional iron immediately available for erythropoiesis and provides direct information on iron-deficient erythropoiesis over the previous period of 3-4 days [6,7]. Reticulocyte hemoglobin content presentedas a good predictorfor the absenceof bone marrow iron stores in patients without macrocytosis, i.e. MCV<100fl[31].Its reduction indicates iron-deficient erythropoiesis, even in conditions like inflammation or ACD, when traditional biochemical markers such as ferritin and TSAT are inadequate [9]. Reticulocyte hemoglobin content showsa high sensitivity and specificity in the diagnosis of IDA [10] and efficiently differentiatesIDA and ACD patients [38]. The clinical utility of CHras a reliable indicator of functional ID has been well established in chronic kidney disease and in hemodialyzed patients [36]. Biochemical parameters have shown limitations in the evaluation of iron status in this group of patients, because of inflammatory activity.The biomarker indicates early iron-restricted erythropoiesisin patients treated with ESAs as they may have functional ID and respond to iron therapyeven with very high serum ferritin values [25]. It also provides an early measure of the response to iron therapy, increasingwithin 2–4 days of the initiation of intravenous irontreatment [5]. Studies for determining intravenous iron replacement therapy in similar patient groups indicate CHr as a more accurate biomarker for predicting functional ID than ferritin and TSAT, andreduces the intravenous iron exposure [5,8]. In CKD patients treated with ESAs, CHr presents as a reliable test that can assess whether the available iron is sufficient for stimulated erythropoiesis. Responders to intravenousiron therapy are better identified when combining percentage of hypochromic erythrocytes and CHr [43].

Some diagnostic limitations are typical for CHr, for it is calculated from the cell volume and its reduction reflects impaired hemoglobin production. It is not suitable for assessing the adequate presence of iron in microcytic anemias such as thalassemia, where CHr is low[7,31]. Reticulocyte hemoglobin content is reduced in other microcytic anemias and increased in megaloblastic anemia and drug-induced macrocytosis as hydroxyurea treatment. The valuesshould be interpreted in the context of patient-specific erythrocyte physiology, information on recent blood transfusions, iron therapy, vitamin B12 or folate deficiency, chemotherapy and hemoglobin electrophoresis results [36].

Hypochromic red blood cells

Erythrocytes are produced continuously by the bone marrow, but in case of prolonged ID with depletion of iron stores and iron-deficient erythropoiesis, cells with low hemoglobin concentration– hypochromic erythrocytes, are produced. Because ofthe long life span of RBCof approximately 3 months, several populations of normochromic and progressive hypochromic cells are available in the peripheral blood[46].The percentage of hypochromic erythrocytes(%Hypo) provides additional information about the iron status and ID over the past 2-3 months and isa late biomarker for iron-deficient erythropoiesis[3,21,46].As this parameter reflectsmore accurately the concentration of hemoglobin in individual cells rather than the meansuch as MCH or mean cellhaemoglobin concentration (MCHC), it is a more sensitive-marker because small changes in the number oferythrocytes with inadequate hemoglobin can be measuredbefore any change in the MCHCoccurs [5].In anemic patients treated with ESAs, particularly anemia of CKD or patients on hemodialysis, %Hypo is useful in identifyingiron-restricted erythropoiesis. As ESAs response depends on iron availability and is limited by iron deficiency - absolute or functional, %Hypo can optimize the response after intravenous iron treatment [3, 8, 43]. Both CHr and%Hypo differentiate efficiently IDA and ACD, revealingwhether iron is not available for erythropoiesis, but not whether ironavailability is restricted, or if there is absolute iron deficiency in abone marrow iron controlled study [38]. Percentage of hypochromic erythrocytes reflects the iron balance for a longer period

than CHr due to the long lifespan of erythrocytes and is less sensitive than CHr for IDA and ACD diagnosis and monitoring of iron treatment. These two biomarkers shouldbe combined with a blood count analysis [38]. Hypochromic erythrocytes bare the same diagnostic limitations as CHr.

Parameters reported by other automated analyzers using the flow cytometric method have similar informative value for erythropoiesis to CHrand Hypo of the ADVIA analyzer (Siemens Healthcare Diagnostics, USA). These are reticulocytes hemoglobin equivalent (RetHe) and hypochromic RBC (HypoHe)bySysmexXE (Sysmex Corp, Japan), mean cell hemoglobin of reticulocytes (MCHr) and hypochromic RBC (%HPO) by CELL-DYN Sapphirre (Abbott Diagnostics, USA), and other[36,46].These new parameters are exclusive of each manufacturer as each company applies the technology in a different way in the analyzers [46]. These lead to other limitations for both biomarkers, related to the lack of standardization, establishment of reference values and diagnostic thresholds, because they vary according to the manufacturer and pose difficulties to compare results [5, 9, 36].

Conclusions

Biomarkers of tissue ID and hypochromia expand information on iron status and RBC production at tissue and cellular level. Although still problems exist with standardization and comparison, the diagnostic role of these new biomarkers has been mentioned in the guidelines on the diagnosis of ID and anemiain chronic inflammatory diseases and medical conditions. They reflect iron supply to erythroblasts for hemoglobinization and iron-deficient erythropoiesis in complex clinical situations and may provide help for identifying ID and differentiate anemia in clinical practice.

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Прогностичното влияние на съотношението Неутрофили към Лимфоцити при лечението с Nivolumab на авансирал недребноклетъчен белодробен карцином: преглед на литературата

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The Neutrophil to Lymphocyte Ratio as a prognostic marker in the treatment of advanced non small cell lung cancer after treatment with Nivolumab: review of the literature

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РЕЗЮМЕ:

Търсенето на прогностични маркери в лечението на авансиралия недребноклетъчен карцином на белия дроб (НДКБД) без доказани активиращи онкогенни мутации е основна задача на много изследователи в последните няколко години. Въвеждането на нови, скъпоструващи терапевтични възможности, както имунотерапията например, прави нуждата от правилна селекция на болните още по-необходима и належаща. Високата изходна стойност на възпалителния маркер NLR- съотношение неутрофили/лимфоцити, има доказана негативна роля по отношение на преживяемостта на пациентите и немалко автори го определят като надежден сурогат за предварителен подбор при избора на лекарствено лечение при НДКБД. С активното приложение на имунотерапията като изключителна важна част от терапевтичната стратетия на пациентите с нелечим белодробен карцином ролята на инфламаторния индекс става все по-интригуваща.

ABSTRACT

Many investigators are looking for reliable prognostic markers in the treatment of advanced non-small cell lung cancer (NSCLC) without activated oncogene mutations. New, expensive drugs, like the immunotherapy, are changing the paradigm of the lung cancer treatment and thus better selection for patients who would benefit most is extremely needed. High pretreatment Neutrophil to Lymphocyte Ratio (NLR) is a negative prognostic marker in terms of survival in many patients with solid tumors and is thought to be a reliable, readily available and cheap method in routine clinical practice. Since many patients with advanced NSCLC at some point of their treatment are suitable candidates for a therapy with immune checkpoint inhibitors the role of the inflammatory index in determination most benefit is extremely challenging.

Ключови думи: недребноклетъчен белодробен карцином, прогностичен маркер, съотношение неутрофили/лимфоцити

Key words: non small-cell lung cancer, prognostic marker, Neutrophil to lymphocyte ratio

Introduction

Lung cancer represents an important social disease, as it continues to be the most frequent cause of cancer related death worldwide and often affects actively working people. Non-small cell lung cancer (NSCLC) accounts for nearly 85 % of all cases; most patients are unfortunately diagnosed in advanced metastatic setting. In that case anticancer treatment is of primary importance and its aim is longer survival of the patients with good quality of life. Despite the therapeutic progress made in the last decades patients with advanced NSCLC rarely live longer than 12 months (1). Platinum-based chemotherapy remains the first line treatment for the majority of patients without targetable oncogenic driver alterations with disease control rate rarely exciding more than 8-9 months. Until recently, at progression very few second line options were available with moderate activity and consisted mainly of single agent chemotherapy ± anti-angiogenic agents or tyrosine-kinase inhibitors with response rate around 10% (2-5).

Anti-programmed-death-receptor-1 (PD-1) antibodies block the interaction between PD L1 receptor on the T-cells and its ligand PD-L1, expressed usually on tumor cells (6). Recently, three major phase III clinical trials have shown superiority of the two anti-PD-1 antibodies Nivolumab and Pembrolizumab with regards of overall and progression free survival, response rate and quality of life compared to standard second line chemotherapy with Docetaxel (6-8). However, immunotherapy is expensive and only few patients – around 20% of all cases, experience long-term benefit and until now no reliable prognostic and predictive markers for response and survival have been detected. Expression of the PD-1 ligand on tumor cells or immune cells determined by immunohistochemistry may be accepted as a prognostic marker only for Pembrolizumab but its use is limited due to dynamic changes over time, intra-tumoral heterogeneity, different test methods with varying thresholds and subjective evaluation of method. Furthermore, no association of PD-L1 expression and benefit from Nivolumab treatment in squamous cell NSCLC as detected. Other potential biomarkers as high mutational burden is still under evaluation with promising initial results bit the price and the time for its detection are a clear obstacle in the routine practice (9).

Inflammation is a known major driver for the development and progression of cancer . The presence of immune cells in the tumor environment, mainly CD8 T cells, is associated with better prognosis for the patients. On the other hand, neutrophils, M2 macrophages and FOXP3 positive regulatory T cells play an important role in tumor progression and resistance to the treatment (10-12). The Neutrophil to Lymphocyte Ratio (NLR) is a reliable negative prognostic market in several cancer types, including advanced NSCLC in the pre-immunotherapy era. Its evaluation is easy, standardized, and cheap. The opportunity to apply the NLR as a prognostic and predictive biomarker in patients with advanced NSCLC, treated with such expensive drugs as immunotherapy, is challenging as the ratio would determine that subpopulation of patients with no benefit under this therapeutic strategy.

This report focuses on two different retrospective clinical trials with patients with advanced NSCLC treated with Nivolumab after failure of first-line chemotherapy. The hypothesis of both studies is that patients with initially high NLR progress rapidly and live shorter.

Patients and methods

The study of Diem S et al (13), published in February 2018, analyses 52 patients with advanced NSCLC, who after progression of first line chemotherapy have received second or subsequent line of therapy with Nivolumab. The patients have been followed for 14 months (05.2015-06.2016) and have received at least one infusion of Nivolumab at a standard dose of 3 mg/kg every two weeks. All patients were ECOG PS 0-1, with adequate organ functions and blood tests and with no evidence for any human immunodeficiency virus or hepatitis, use of systemic steroids, concomitant radiotherapy

or previous or ongoing autoimmune disorder. Before starting treatment with Nivolumab NLR was calculated by division of absolute Neutrophil and lymphocyte counts measured in peripheral blood. The primary endpoint of Diem S et al is the correlation between the high pretreatment value of NLC and the overall and progression free survival (OS, PFS). For descriptive statistics patients were subdivided into three groups defined by the tertiles of NLR: NLR<3.6, 3.6<NLR<6.5 and NLR>6.5. The patients in every group are equally distributed - 17,18,17, respectively. The second endpoint of the study is the correlation between particular demographic (PS, age, smoking status, sex, histology, number of metastatic sites, metastatic site) and pretreatment laboratory characteristics (LDH, CRP, albumin) and the prognosis of the patients.

The study of Park W et al (14) have created a retrospective database of 159 patients with advanced NSCLC, who have received second and further treatment with Nivolumab. The authors have tried to create a descriptive clinical model to distinguish the patients with potential benefit of the immunotherapy. They call it iSEND model and it consists of several parameters – the application of immunotherapy, ECOG PS, the pretreatment value of NLR and Delta NLR (the ratio between the neuthophils and the result of subtraction between leucocytes and neutrophils). Patients are categorised into three groups according to iSEND value and an association between these groups and the prognosis of the patients (in terms of PFS and OS benefit) has been evaluated. Additional task of the project is the correlation of the NLR itself and survival rate of the population.

Results

Table 1 summarizes the patients` and tumor characteristics according to the study of Diem S et al:

Table 1: ***Patients` and Tumor characteristics, Diem S. и сътр. (13)***

Age	66.2 (45.5-88.2)
Sex	
Male	29 (56%)
Female	23 (44%)
Smoking status	
Current smoker	21 (40%)
Former smoker	27 (52%)
nonsmoker	4 (8%)
ECOG PS	
0	13 (23%
1	32 (65%)
2	7 (12%)
Metastatic site	
Bone	17 (33%
Liver	17 (33%)
Lung	15 (29%
Brain	15 (29%
Adrenal glands	10 (19%
Pleura	9 (17%)
Soft tissue	6 (12%)
Other	9 (17%

Number of metastatic sites	
1	13 (25%)
2	15 (29%)
3	12 (23%)
4	6 (12%)
5	4 (8%)
>5	2 (4%)
Histology	
Adenocarcinoma	30 (58%)
Squamous	22 (42%)
NLR	5.0 (2.7-8.3)
Platelets (g/l)	256 (227-341)
CRP (mg/l)	26 (5-78)
LDH (U/l)	246 (199-322)
Albumin (g/l)	33.8 (28.7-36.8)

The majority of patients in the study of Diem S et al have adenocarcinoma (58%), are former or active smokers (92%), and have bone (17%) and/or liver metastases (17%). The median NLR is 5 (2.7-8.3).

For the whole group of patients included in the analysis median OS and PFS are 9.6 months (95% CI 6-14) and 2.1 months (CI 95% 1.8-6.4), respectively. Higher NLR is associated statistically significant with worse OS (p=0.013) and there is a trend towards shorter PFS (p=0.114). When dividing the population into three groups according to tertiles of NLR – NLR<3.6; 3.6<NLR<6.5; NLR>6.5, the median OS in each group is 15.3 months, 13.15 months and 4.69 months, respectively – **table 2.** The multivariate analysis detects a strong and statistically significant association between the pre-treatment NLR and OS – **figure 1.**

Table 2: ***Overall survival and pre-treatment NLR according Diem S et al (13):***

		Number patients	Median	p
Overall survival	NLR<3.6	17	15.3 months	p=0.001
	3.6<NLR>6.5	18	13.15 months	
	NLR>6.5	17	4.69 months	

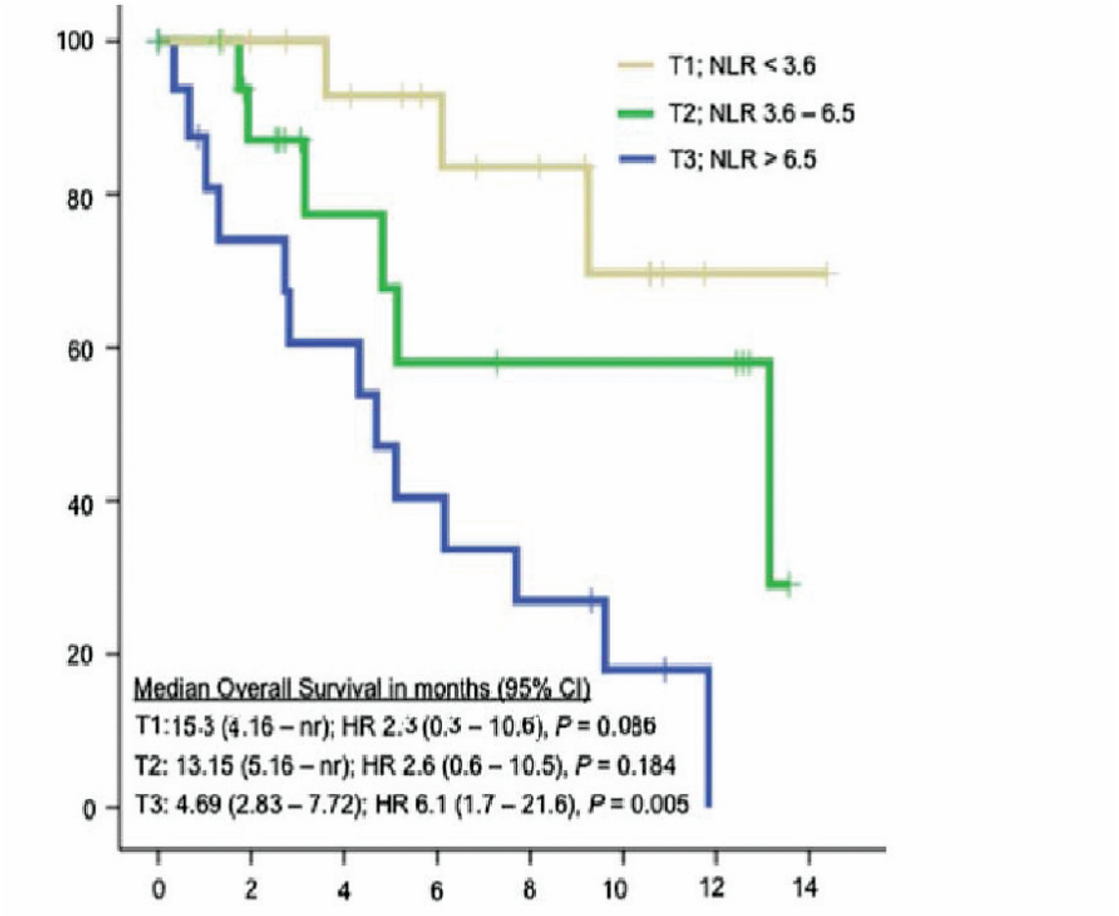


Fig. 1 ***Overall survival according to NLR tertiles, Diem S et al.***

In the multivariate analysis no correlation between the demographics characteristics of the patients and the pretreatment NLR value is detected. Only the high value of CRP is associated with the inflammatory ratio – p=0.02, but is available in 19 patients only.

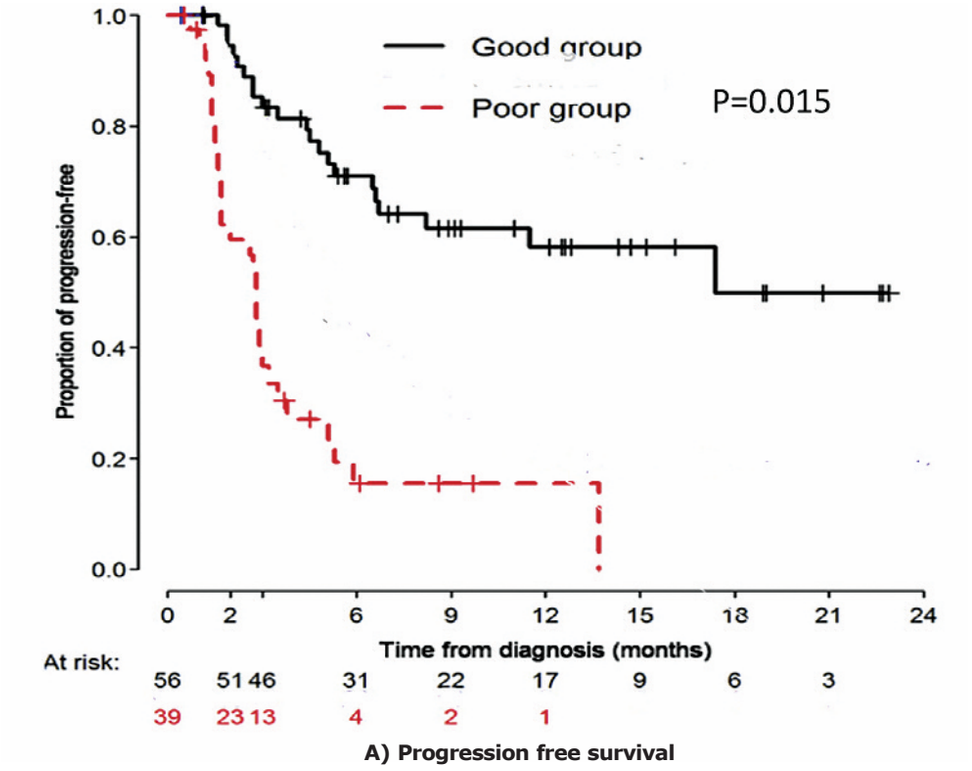
The study of Park W et al (14) evaluates 159 patients between Mar 2015-Mar 2017, and at the beginning the dose of Nivolumab is 3 mg/kg. After the FDA approval of the standard fixed dose of 240 mg every 3 weeks the dose of the checkpoint inhibitor has been altered. Quite an interesting matter is that in this trial 13 patients have been with oncogenetic driver mutations – either EGFR activation mutations or ALK rearrangements. In the study patients have been divided into two groups based on the pretreatment NLR value - ≥5 and <5, respectively. The primary endpoint of the project is the association between iSEND model, NLR and the prognosis of the patients; the second endpoint is the relation between some of the demographic characteristics of the patients and the potential prognostic markers (NLR and iSEND) – **table 3.**

Table 3: ***Demographic characteristics of the patients according to Park W et al (14):***

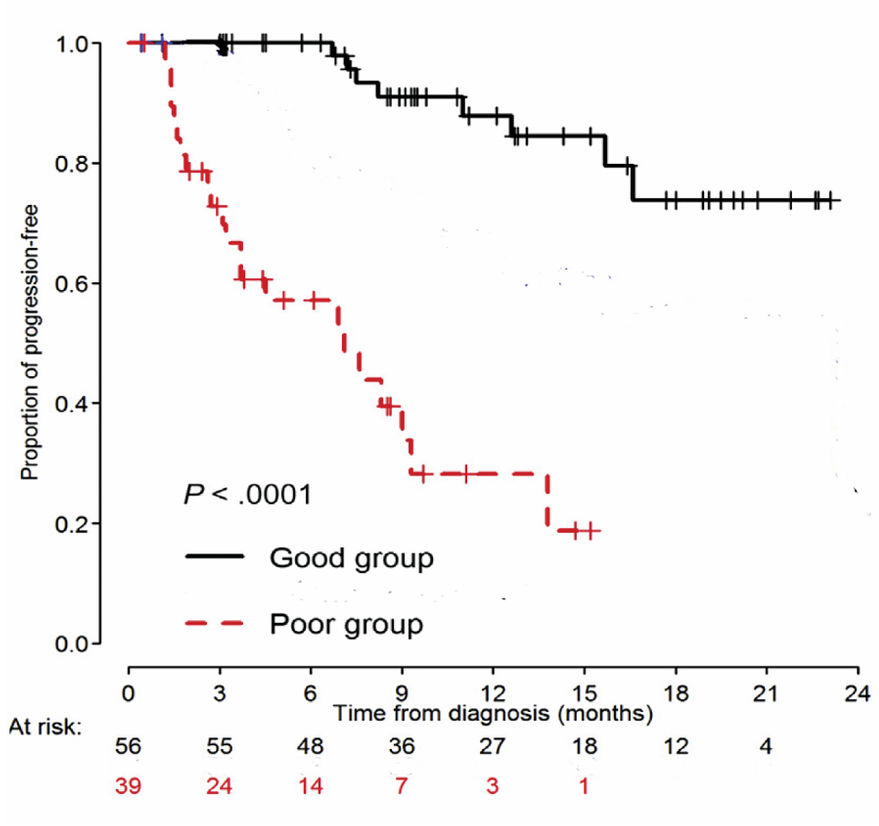
Age	68 (41-91)
Sex	
Male	82 (52%)
Female	75 (48%)
Smoking status	
Current smokers	73 (45.9%)
Ex smokers	37 (23%)
Nonsmokers	49 (31.1%)
ECOG PS	
0	10 (6%)
1	89 (55%)
2	60 (39%)
Histology	
Adenocarcinoma	109 (68.6%)
Squamous	39 (24.5%)
other	12 (7%)
NLR	4.3 (0.5-24.1)

The majority of the patients are male, ex or current smokers, in good performance status (ECOG PS 0-1) and with adenocarcinoma. The median previous lines of treatment is 1 (range 1-6), only 15% of the population have experienced more than two subsequent treatment regimens.

The results of this study detect that male patients (HR 1.91; 95% CI 1.25-2.93, p=0.002) and poor performance status, ECOG PS 2 (HR 2.1; 95% CI 1.28-3.34, p=0.002) correlates statistically significant with worse PFS independently; for OS the significance is not statistical. Patients with NLR≥5 (poor prognostic group) progress earlier (worse PFS, HR 1.68; 95% CI, 1.11-2.54, p=0.015) and live shorter (worse OS, HR 2.5; 95%CI, 1.05-2.89, p<0.0001). Park W et al. conclude that during treatment with Nivolumab patients with elevated pretreatment inflammatory marker have poorer prognosis despite the immunotherapy. The median NLr value for the whole population in the trial is 4.6 (0.7-22.1). Additional contribution of the study is the evaluation of the change the NLR during therapy – if NLR remains elevated, over 5, patients have significantly shorter PFS and OS - p=0.005 and p<0.0001q respectively – **fig 2.**



А) Progression free survival



Б) Overall survival

Fig. 2 ***Kaplan Meier Curves for Progression free survival А) and Overall Survival Б) according to Park W (14) et al in terms of the pretreatment NLR value (good prognostic group NLR<5, poor prognostic group - NLR≥5.***

Discussion

The ant-PD-L1 antibody Nivolumab has been recently approved in NSCLC as second-line therapy after failure of platinum-based chemotherapy; still reliable biomarkers have not been detected. Despite its superiority to standard chemotherapy, less than 20% of all patients treated with checkpoint inhibitor experience disease control rate (complete, partial response or stable disease) after 2 years of therapy; progression of the disease occurs in all other patients (15). Achieving a durable response and maximal clinical benefit from PD-1 inhibition requires a couple of key host-tumor immunologic components, which include immunogenic tumor, reversible immune exhaustion, favorable host immune balance and effective anticancer immune reactivation (15-19). The aggressive biology of NSCLC, the high price of immunotherapy and the relatively small percentage of patients with persistent benefit from checkpoint inhibitors as second line treatment demonstrates the need of reliable biomarkers for better selection of patients.

In recent years the inflammatory index Neutrophil to lymphocyte ratio proved to be a reliable prognostic biomarker for many solid tumors, including advanced NSCLC. Many retrospective studies focus on the use of NLR for the determine prognosis of patients with NSCLC under chemotherapy treatment; a relation between its pretreatment value (before starting therapy) and progression free and overall survival has been under evaluation (20-24). The conclusions so far have shown that patients with high value of the inflammatory marker (usually NLR≥5) respond less to chemotherapy and progress earlier than patients with normal (NLR<5) value.

Immunotherapy is proving to be an essential therapeutic option for many patients with advanced NSCLC. To optimize the treatment, to find that subpopulation of patients with greatest benefit of the therapy and minimize the huge amounts of money reliable prognostic biomarkers are needed.

In two separate retrospective studies Diem S et al and Park W et al investigate the prognostic for survival (progression free and overall) role of the pretreatment NLR value in patients treated with immunotherapy as second and subsequent line. Both authors demonstrate that the high baseline NLR is significantly correlated with worse OS; Park W et al also prove the same correlation with PFS as well (in their study Diem S et al find only a slight similar trend). The results of both studies support the recent evidence presented by many other publications for the negative prognostic role of high pretreatment NLR in patients with advanced NSCLC, treated with chemotherapy. In a meta-analysis, including 14 retrospective studies with more than 3500 patients with advanced NSCLC, NLR≥5 is associated with worse PFS and OS (24). Another analysis with 325 treatment naive metastatic NSCLC patients shows that despite platinum-based chemotherapy high baseline NLR correlates with poorer survival (25). In addition a high preoperative NLR is an independent negative prognostic indicator in operable NSCLC patients (26, 27). However, there is still limited data about the prognostic role of the inflammatory index in patients treated with immunotherapy. In a very recent publication in „Lung Cancer" association of high pretreatment NLR with poor outcome could be shown in a cohort of NSCLC patients treated with immune checkpoint inhibitors (Nivolumab, Pembrolizumab, Atezolizumab) (28).

Regarding some of the demographic characteristics of the patients, evaluated by Diem S et al and Park W et al, only poor performance status and male sex are associated with significantly worse prognosis; the other factors including the tumor volume described by the TNM classification fail to show any significant correlation. The results of both studies about the prognostic role of the ECOG PS is essential because in comparison with the chemotherapy treated patients where its value is well recognized in the immunotherapy era the significance of the worse performance status is still not clear (29).

Concerning the biochemical parameters evaluated in both studies only high CRP correlates with worse overall survival (according to Diem S et al, not proven by Park W et al); LDH, albumin level and platelets do not play a prognostic role.

Both studies have several limitations. Firstly, the retrospective nature of the investigations compromises the selection of patients, which often could be biased. Secondly, all treated with Nivolumab patients have received their therapy due to an early access program, thus the treating physicians were obliged to follow the stringent in- and exclusion criteria for Nivolumab therapy. Patients on corticosteroids or patients who have received radiotherapy in the last 14 days despite the reason (pain, brain mets, vena cava syndrome) were excluded from the studies because of the risk of false elevated blood parameters. Thirdly, the number of the involved patients in both trials is small and the role of NLR is evaluated only in these who have progressed on first line therapy. Therefore, their baseline immunity might have been affected by the previous chemotherapies although the number of lines of previous therapy did not correlate with clinical outcomes. The dose of Nivolumab, used in both studies, is different: Diem S et al focused on Nivo 3mg/kg, while Park W et al have started initially with the same dose and afterwards have changed it to fixed dose of 200 mg. And last but not least there are some essential differences in the design of the two clinical trials which could have also biased the results. The authors of the first study exclude patients with activating EGFR or ALK mutations, while Park W et al include them. The stratification of the inflammatory index also differs between the two working groups – Diem S et al assume the trichotomy division of NLR, while Park W et al – the dichotomy; again, this difference could compromise the results. In the second study patients progressed on second and further lines of therapy have been included, while in the first one patients have received mmunotherapy only as a second line. And lastly, Park W et al focus not only on the prognostic but also on the potential predictive role o NLR; the authors examine whether the normalization of the maker correlates with better prognosis for the patients treated with immunotherapy.

Conclusion

High pre-treatment NLR is a simple and reliable prognostic marker which strongly correlates with poor survival in NSCLC patients treated with the anti-PD1 antibody nivolumab. NLR is cheap and readily available biomarkers adding additional prognostic information to identify patients benefiting from treatment with nivolumab. Considering the limitations of both abovementioned studies these results may be helpful in counselling patients before commencing treatment with immunotherapy. Further prospective studies with adequate sample sizes are needed to validate the results and define NLR as an applicable prognostic marker in the immunotherapy era.

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Място и значимост на ангионевротичния оток в структурата на токсико-алергичните реакции и общата алергична патология в Отделението по професионални заболявания с дейност по алергология, УМБАЛ Св. Георги, Пловдив

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Place and significance of angioedema in the structure of toxic-allergic reactions and general allergic pathology in the Occupational Diseases and Allergology Ward, UMHAT St. George, Plovdiv

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РЕЗЮМЕ:

Въведение: Това проучване има за цел за изследва мястото и значението на ангионевротичния оток в структурата на токсико-алергичните реакции и общата алергична патология. По-точно, интересува ни честотата на ангионевротичния оток срез пациенти, хоспитализирани за токсико-алергични реакции, както и на най-често срещаните етиологични фактори за отока. *Материал и методи:* Данните за настоящето проучване са получени от официална медицинска документация и регистри. Използвахме информация за пациентите, хоспитализирани от 2015 до 2017 в Отделението по Професионални болести с дейност по алергология на УМБАЛ Св. Георги, Пловдив. *Резултати и обсъждане:* Намерихме тенденция към повишаване на абсолютната честота на ангионевротичен оток, както и повишен дял на случаи с ангионевротичен оток в сравнение с други алергични заболявания. Най-честите провокиращи фактори са лекарства, хранителни алергии, насекоми и други неизвестни фактори. Въпреки относително малкото случаи на наследствен ангиооток и професионално-обусловен ангиооток, те са от особен интерес предвид специфичното им протичане, етиология, патогенеза, клинична картина, диференциална диагноза и трудово-медицинска оценка. Заключение: Ангионевротичният оток е заболяване с различни провокиращи фактори и нарастваща честота в проучването ни. Чрез задълбочаване на разбирането ин за епидемиологията, етиологията, патогенезата и клиничната му картина, диагностичните и терапевтични възможности, налични на клиницистите, ще се повишат.

Ключови думи: алергични заболявания; ангионевротичен оток; клинична пътека; токсико-алергични реакции

ABSTRACT

Background: This study aimed to explore the place and significance of angioedema in the structure of toxic-allergic reactions and general allergic pathology. More specifically, we were interested in the prevalence of angioedema among patients hospitalized for toxic-allergic reactions, as well as the most common etiologic risk factors for angioedema. *Material and methods:* Data for the present study were obtained from official medical records and registries. We elicited information on patients hospitalized from 2015 to 2017 in the Occupational Diseases and Allergology Ward at UMHAT St. George, Plovdiv. *Results and discussion:* We observed an upwards trend toward higher absolute prevalence of angioedema, as well as higher proportion of cases with angioedema relative to cases of other allergic diseases. The most common triggers for angioedema were medication use, food allergies, insects, and other unknown factors. Although relatively few cases of hereditary angioedema and work-related angioedema were observed, those are of particular interest given their idiosyncratic etiology, pathogenesis, clinical presentation, differential diagnosis, and work-related assessment. Conclusion: Angioedema is a disease characterized by various triggers and an increasing prevalence in our study. By deepening our understanding of its epidemiology, etiology, pathogenesis, and clinical presentation, the diagnostic and adequate therapeutic options available to clinical practitioners are expected to increase.

Key words: Allergic diseases; Angioedema; Clinical pathway; Toxic-allergic reactions

Introduction

Edema is a poly-etiologic syndrome characterized by diverse underlying pathophysiologic mechanisms leading to pathological retention of fluid in the interstitium (13). Vascular permeability is increased under the influence of the released vasoactive substances, which further relates to an efflux of fluid in the extravascular compartment. Angioedema is one of the subtypes of this syndrome. It is characterized by a sudden and pronounced swelling of underlying derma and hypoderma, accompanied by a painful sensation, and to a lesser extent by itching; it oftentimes involves the mucosae and tends to remit slower compared to urticaria (16). HAE is a subtype of angioedema. It is considered a rare disease pertaining to the rare immunodeficiencies of the complement system proteins (5, 12, 18). Albeit rare, HAE had been linked to an increased mortality risk due to laryngeal edema (5, 10).

Unlike urticaria, angioedema involves deeper skin layers and is rarely itchy. It either presents in isolation, or alongside other clinical skin/mucosal symptoms, which oftentimes prompt hospitalization of patients with toxic-allergic reactions (16, 19, 20). According to various authors and guidelines, angioedema is associated with urticaria, therefore it should be noted that the latter is distinguished by quickly appearing hives and/or angioedema (5, 9, 12, 21, 22). Urticaria plaques affect the superficial skin layer and are typically accompanied by itching and erythema. There are also differences in the etiology of urticaria and angioedema (2-4, 6-8, 21, 22, 24, 25).

This study aimed to explore the place and significance of angioedema in the structure of toxic-allergic reactions and general allergic pathology. More specifically, we were interested in the prevalence of angioedema among patients hospitalized for toxic-allergic reactions and well as the most common etiologic risk factors for angioedema.

Material and methods

Data for the present study were obtained from official medical records and registries. We elicited information on patients hospitalized from 2015 to 2017 in the Occupational Diseases and Allergology Ward at UMHAT St. George, Plovdiv. The Ward is certified by the Ministry of Health to carry out diagnostic and therapeutic procedures in the field of clinical allergology and occupational diseases. Two thirds of the staff physicians have at least two clinical specialties, two are allergologists, three are specialists in occupational diseases, and four are specialists in internal diseases.

The clinical pathway algorithms adhered to the established regulations in Bulgaria (14, 16, 17, 19, 20, 24). The two clinical pathways we focused on were № 291 „Toxic-allergic reactions in individuals older than 18 years" and №106.1 „Diagnosis and treatment of toxic-allergic reactions" in patients older than 18 years (19). The diseases covered in these pathways were coded according to the tenth revision of the International Classification of Diseases (ICD-10) (15).

The prevalence of cases with angioedema is expressed as the percentage from all patients treated for allergic diseases during the respective time period. Results are presented in tables and figures to enhance readability. The interpretation of these results and final diagnoses indicated in patients' medical records were done by specialists and follow the established medical standards, criteria, and requirements (17, 20).

Results

Table 1 shows the number of patients hospitalized in the clinic for angioedema in 2015, 2016 and 2017. Results indicate that there is a continuous increase in those hospitalizations. For 2017, we only had data covering the first six months of the year. That makes our results conservative and, given the incident cases of angioedema, reinforce the notion of an upwards trend in the number of cases with angioedema.

Table 1. ***Number of patients hospitalized in the clinic for angioedema in 2015, 2016 and 2017***

Year	Clinical pathway (№)	All patients (N)	Patients with angioedema (N)	%
2015	291	272	39	14.34
2016	291	50	8	16.00
2016	106.1	165	33	20.00
2017	106.1	125	13	10.40

Table 2 shows the number of patients hospitalized in 2015 stratified by clinical pathway and month. Out of the 272 patients, 39 were diagnosed with angioedema, accounting for 14.34 % of all patients.

Table 2. ***Number of patients hospitalized in 2015 stratified by clinical pathway and month***

Clinical pathway (№)	Month												N
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
92	-	1	1	2	1	1	1	-	1	-	1	-	9
94	13	17	19	18	18	10	9	9	11	8	15	11	158
290	17	7	-	-	-	-	-	-	-	-	-	-	24
291	21	20	21	23	27	28	16	27	20	26	20	23	272
Total number of patients													463

Table 3 shows the number of patients hospitalized in 2016 stratified by clinical pathway and month. Out of the 215 patients (clinical pathways 291 and 106.1), 41 were diagnosed with angioedema, accounting for 19.07 % of all patients.

Table 3. ***Number of patients hospitalized in 2016 stratified by clinical pathway and month***

Clinical pathway (№)	Month												N
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
92	-	-	1	-	-	-	-	-	-	-	-	-	1
40.1	-	-	-	1	-	2	-	-	-	1	-	-	4
94	9	28	27	2	-	-	-	-	-	-	-	-	66
41.1	-	-	-	27	18	26	3	6	8	9	6	9	112
291	31	19	-	-	-	-	-	-	-	-	-	-	50
106.1	-	-	-	-	-	17	18	23	26	24	31	26	165
Total number of patients													398

Table 4 shows the number of patients hospitalized in 2017 stratified by clinical pathway and month. Out of the 125 patients (clinical pathway 106.1), 13 were diagnosed with angioedema, accounting for 10.4 % of all patients.

Table 4. ***Number of patients hospitalized in 2017 stratified by clinical pathway and month***

Clinical pathway (№)	Month												N
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
40.1	-	1	-	1	-	5	2	11	10-	9	11	9	59
41.1	12	19	15	13	12	23	12	-	-	-	-	-	106
106.1	20	24	24	31	24	2	-	-	-	-	-	-	125
Total number of patients													290

Figure 1 shows the frequency of different etiologic factors that triggered angioedema in the period 2015 – 2017. In the majority of patients in 2015 – 2017, angioedema was triggered by mediation intake. Moreover, it seems that the relative share for mediation intake has increased over the years. Other triggers include food and insects, followed by cases with mixed or unknown etiology. Compared with other factors, occupational exposure to allergens seems less important. In 2015, we observed several cases of hereditary angioedema.

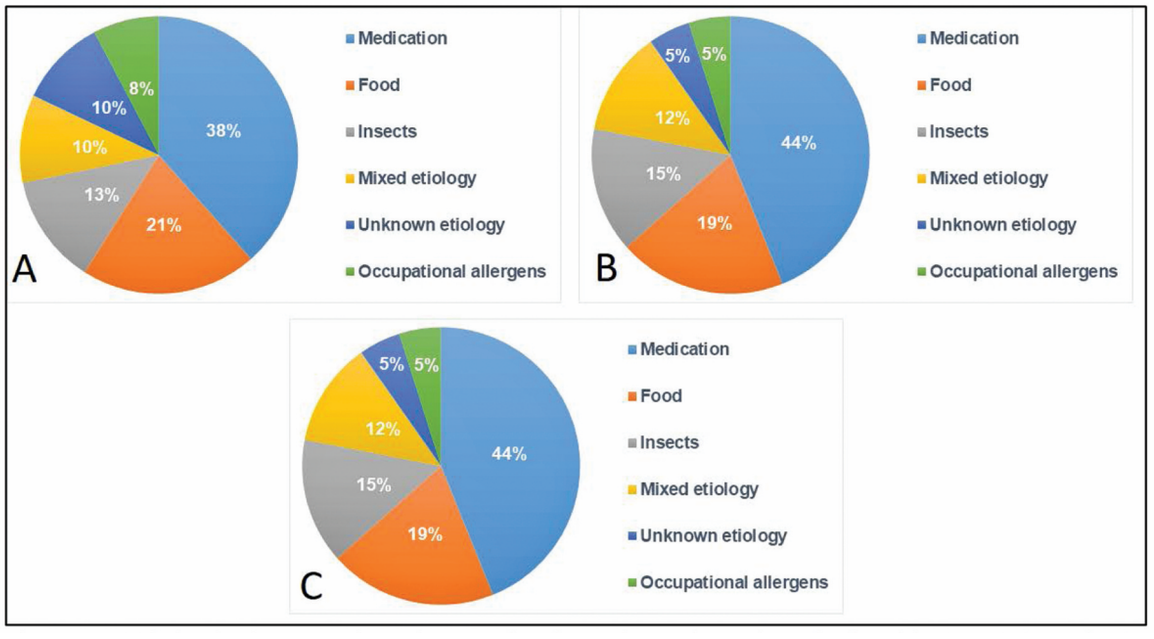

Figure 1. ***Etiologic factors triggering angioedema in patients hospitalized in 2015 (Panel A), 2016 (Panel B), and 2017 (Panel C)***

Discussion

In the present study, we explored the prevalence of angioedema among patients hospitalized for toxic-allergic reactions and well as the most common etiologic risk factors for angioedema. We observed an upwards trend toward higher absolute prevalence of angioedema, as well as higher proportion of cases with angioedema relative to cases of other allergic diseases. The most common triggers for angioedema were medication use, food allergies, insects, and other unknown factors. It is not surprising that around 40% of cases were triggered by medication use given

that different pharmacological groups can activate different immune or non-immune (pseudo-allergic) mechanisms of extravasation and (3, 6-9, 23). In addition, on some occasions the actual medication that has cause the attack cannot be established (11).

The same can pretty much be said of food allergies. As for other triggers for angioedema, the results were somewhat unexpected (i.e., the high prevalence of insect-related angioedema). Although relatively few cases of HAE and work-related angioedema were observed, those are of particular interest given their idiosyncratic etiology, pathogenesis, clinical presentation, differential diagnosis, and work-related assessment.

The concept „clinical pathway" represents a system of instructions and requirements for algorithm that medical specialists should follow in the process of diagnosis and treatment of patients with specific diseases that require hospitalization in healthcare facilities with a stationary (17). Our study focused on patients treated according to pathways № 92/40.1, 94/41.1, and 291/106.1. We covered a wide spectrum of allergic diseases. Despite the fact that some conditions, including allergic rhinitis, urticaria, and HAE are not included in these clinical pathways, Bulgarian regulations allow for patients with those diseases to be diagnosed and treated (1, 9, 14, 16, 17, 19, 20, 26). Over the study period, there have been some changes in the clinical pathway coding, but not in the taxonomy and nomenclature of the respective diseases and diagnostic/treatment algorithms.

We acknowledge that, for 2017, we were able to include patients hospitalized up to the end of June. However, that only reinforces our hypothesis that the prevalence of angioedema is rising. Further, it was beyond the scope of this study to investigate the group of „unknown" triggers, which would require in-depth tests on case-by-case basis.

Conclusion

Angioedema is a disease characterized by various triggers and an increasing prevalence in our study. By deepening our understanding of its epidemiology, etiology, pathogenesis, and clinical presentation, the diagnostic and adequate therapeutic options available to clinical practitioners are expected to increase.

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Колко многолика може да бъде клиничната изява и етиология на алергичните болести: Съчетание на алергични болести с разнообразна клинична изява, включително професионална

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How multifaceted the clinical presentation and etiology of allergic diseases could be: Concomitant allergic diseases with diverse clinical presentation and etiology, including work-related

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РЕЗЮМЕ:

Алергичните заболявания при работещите в хлебопроизводството не са изолирано явление. Все още, обаче, в специализираната медицинска литература не е даден ясен отговор на въпроса защо у едно и също лице могат да се съчетаят няколко алергични болести, какви са етиологичните фактори и патогенетични механизми довели до тяхното възникване и комбиниране. Представеният от нас случай допълва познанията за пъстрия спектър на алергичните болести, чиято етиология и клинична изява у един и същ пациент могат да доведат до уникално съчетание и протичане.

Ключови думи: алергични болести, професионална експозиция, хлебопроизводство

ABSTRACT

Allergic diseases in bread production workers are not uncommon. However, the medical literature has not yet provided a clear answer as to why an individual may suffer concomitant allergic diseases, and what etiological factors and underlying mechanisms may have led to their onset. The present case report helps fill this gap in the literature on the broad spectrum of allergic diseases, whose etiology and concomitance in a patient may lead to a unique clinical manifestation.

Key words: Allergic diseases; Occupational exposure; Bread production

Introduction

The role of job title and occupational environment in the onset and progression of allergic diseases has been extensively studied in Bulgaria [9, 22]. Job title and occupational exposure to various substances, acting as complete or incomplete antigens (haptens), have been discussed as important factors in allergic disease etiology and pathogenesis [22, 25, 26]. Other key issues in the field are the joint effect and interaction of these factors [2, 10, 25, 26, 31]. Concomitance of asthma and rhinitis has been proven and is thoroughly-studied [2, 7, 9, 14, 15, 25, 28]. Other authors report an association between asthma and allergic rhinitis/conjunctivitis and urticaria/dermatitis in selected populations and age groups [6, 16, 13]. There have been multiple reports on concomitant urticaria and angioedema, and some of those served as basis for clinical guidelines established in Europe and Bulgaria [1, 18-20, 25, 28].

In addition to the widely known etiologic factors for allergic diseases, we have previously discussed the role of occupational exposures as a trigger for various toxic-allergic reactions [4, 5, 21, 24]. The clinical presentation of allergic diseases in patients exposed to agricultural goods is quite diverse – raging from the widely known bronchial asthma and hypersensitivity pneumonitis to rhinitis, dermatitis, etc. [26]. There is a wide range of etiologic risk factors for allergic reactions in these individuals. These may or may not be IgE-mediated; as for pathophysiological mechanisms, allergic reactions may develop as hypersensitivity reaction types I, III, or IV, according to the classification of Coombs and Gell [25, 26, 28]. In some cases, other, non-immunologic factors may be involved in the etiology and pathogenesis of allergic reactions, that is genetic, toxic, irritative, physical, and neuro-reflexory factors.

The high prevalence of allergic diseases, especially bronchial asthma, in bread production workers has been associated with exposure to multiple substances, such as protein, enzymes, detergents, and irritants, diverse in their physicochemical composition, sensitizing potential, and mechanisms of action [5, 22, 26].

Development of different allergic diseases in an individual occupationally exposed to allergens from bread production is of interest from both theoretical and scientific point of view. The case we report here is expected to help disseminate the current knowledge on the subject the field foreword.

Material and methods

The source information for this study was elicited from patient's official medical documentation, clinical and paraclinical tests and examination records, as well as photos presented by the patient herself.

Case report

In October 2011, a 41-year-old Caucasian woman was admitted to the Occupational Diseases and Allergology Unit of "St. George" University Hospital, Plovdiv. The patent was consulted and was followed by specialists in occupational diseases and allergology, both prior to and after her hospitalization.

Route to work and anamnesis of occupational risk factors

Patient had 20 years of service. For 6 years, she had been working as a baker in a bread bakery. She had been exposed to wheat flour on a daily basis.

General and focused allergologic anamnesis

Debut of respiratory symptoms was 10 years ago when the patient got first asthmatic attack. In the past few months, the frequency of those episodes of expiratory shortness of breath and wheezing increased, which led to a more frequent use of her personal inhaler. Her condition and symptoms were managed with an inhalable corticosteroid.

Family history

The patient had a family history (her father and sister) of asthma.

Anamnesis of smoking and previous pulmonary diseases

The patient had been an active smoker for around 20 years and smoked 5-6 cigarettes/day.

Anamnestic data and documentation on previous and concomitant allergic diseases in patient's medical record

The patient had congested nose for 15 years, itchy nose and eyes, sneezing, episodes of rhinorrhea and clear secretion by the conjunctivae, and a post nasal drip.

She underwent regular treatment courses with nasal corticosteroids, nasal and oral H1-blockers, and combined medication (antihistamines and decongestants). That resulted only in temporary relief. The allergic nature of her rhinitis and conjunctivitis were confirmed upon consultations with specialists in otorhinolaryngology and ophthalmology.

Urticaria debuted in 2009. The patient suffered a severe allergic episode, which clinically manifested with a generalized urticaria rash and angioedema. In May 2009, the patient was hospitalized at the Toxicology and Intensive Care ward of our University Hospital. Occupational exposure to wheat flour was suspected to had triggered the allergic reaction.

The patient reported aggravation of respiratory, nasal, and skin-mucosa symptoms during work. She had been previous hospitalized for exacerbation of her urticaria.

Complete physical examination

Patients' general status was good. She had clear consciousness, she was alert and afebrile. She had white skin and pale-pink visible mucosae. No lymphadenopathy of palpable lymph nodes was detected. The thyroid gland was not enlarged. Three moderately itching urticaria plaques were detected on the back **(Fig. 1).**

Examination of respiratory system showed symmetric chest, resonant percussion sound, and singular crepitations diffusely spread over both halves of the chest.

Cardiovascular system examination showed regular rate (80 bpm) and rhythm, clear tone, and blood pressure 110 /80 mmHg.

Succusio renalis was negative on both sides (-). Abdomen was soft and non-tender. Liver and spleen were not enlarged. Musculoskeletal system was properly developed for age.

Diagnostic procedures

Tables 1-5 show results of hematological, biochemical, immunological, functional and lung imaging tests, and specific allergic testing.

Table 1. ***Hematological and biochemical tests***

Hematology		Differential blood count		Biochemistry	
Parameter	Reference	Parameter	Reference	Parameter	Reference value
HGB: 129 g/l	120-160	Neut. – 56.6%	50-67	Glucose: 4.5 mmol/l	2.8-6.1
RBC: 4.34 T/l	4.0-5.4	Lymph. – 32.7%	22-45	T. protein: 71.0 g/l	60-83
HCT: 0.392	0.370-0.440	Eos. – 3.3 %	0-6	Albumin: 42.0 g/l	35-55
MCH: 29.8 pg	28-33	Mono. – 4.7%	2-14	Urea: 3.8 mmol/l	2.6-7.2
MCV: 90.4 fl	82-98	Baso. – 0.4%	0-2	Creatinine: 64mkmol/l	44-96
MCHC: 330g/l	300-360			AST-22 U/l	0-36
WBC: 8.00 g/l	3.5-10.5			ALT-19 U/l	0-33
PLT: 374 G/l	140-400			ALP-97 U/l	98-279
ESR: 7 mm/h	2-25			GGT-23 U/l	0-50

Table 2. ***Immunological tests***

Test	Method	Result	Reference value
Tot. IgE	ELISA	**468 IU/ml**	< 100 IU/ml
C3	Nephelometry	0.872 g/l	0,61 – 2,09 g/l
C4	Nephelometry	0.291 g/l	0,122 – 0,495 g/l
C1 esterase inhibitor (Ag)	RID	**583 mg/l**	95-345 mg/l

Table 3. ***Skin allergy tests (Stallergens) using panels of plant and animal allergens***

1. Pollens	20 min.	4. Micro ticks	20 min.
Gr. Tree - 1	(-)	D. farinae	(-)
Gr. Tree - 2	(-)	D. pteronissimus	7/7
Gr. Spring	30/25		
Gr. Summer	15/15		
2. Home dust	15/15	**5. Animal allergens**	
		Cat hair	(-)
		Dog hair	(-)
3. Mold		**6. Controls**	
Gr. Mold allergen 1	25/20	Positive control (histamine)	8/5
Gr. Mold allergen 2	15/15	Negative control	(-)

Skin allergy tests conducted using panels of indoor and outdoor allergens showed polysensitization, mostly to „Spring pollen" and „Mold allergen".

Table 4. ***Spirometry and bronchodilator test***

Indices	Actual values 1	% (ECCS)	Actual values 2	% (ECCS)	Post bronchodilator test (%)
Dynamic					
FVC [L]	3.48	111.1	6.09	117.5	-2.18
FEV 1.0	2.40	89.1	4.49	100.2	+ 14.64
FEV1/VC %	68.99	97.4	73.74	73.74	+ 11.94
PEF [L/s]	5.66	87.6	10.93	108.6	+ 20.1
MEF 25 [L/s]	0.47	26.9	1.94	72.4	+ 24.83
MEF 50 [L/s]	1.74	26.9	3.84	67.9	+ 19.14
Static					
VC [L]	3.64	117.2	6.05	109.4	-2.08

Pulmonary Function Test results showed that asthma was „under control". Bronchodilator test was positive (+), indicating that bronchial obstruction was reversible.

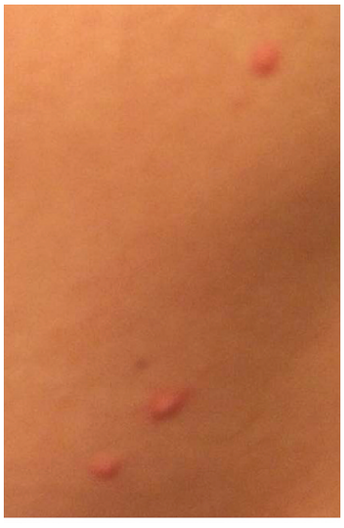

Fig. 1. ***Urticaria rash***

Table 5. ***Epicutaneous test with the European baseline series of epicutaneous tests***

№	*Allergen*	*48-th hour* +/++/+++/++++
1	Potassium dichromate	+
2	4-phenylenediamine base (PPD)	—
3	Thiuram mix	+
4	Neomycin sulfate	—
5	Cobalt (‖) chloride hexahydrate	—
6	Benzocaine	+
7	Nickelsulfate heexahudrate	—
8	Clioquinol	—
9	Colophony	—
10	Paraben mix	—
11	N-Isopropyl-N-phenyl-3-phenylenediamine (IPPD)	—
12	Lanolin Alcohol	+
13	Mercapto mix	+
14	Epoxy resin	+
15	Balsam Peru	—
16	4-tert-Butylphenolformaldehyde resin	—
17	2-Mercaptobenzothiazole (MBT)	—
18	Formaldehyde	—
19	Fragrance mix I	—
20	Sesquiterpene lactone mix	—
21	Qua ternium 15	—
22	2-methoxy-6-n-pentyl-4-benzoquinone (Primin)	+
23	5-chloro-2-methyl-4-isothiazolin-3-one (Kathon CG)	—
24	Budesonide	—
25	Tixocortol-21-pivalate	—
26	Methyldibromoglutaronitrile	—
27	Fragrance mix ‖	—
28	Lyral (alfa-hexyl cinnamal)	+
29	Wheat flour	++
30	Control (reference)	—

Epicutaneous testing followed the guidelines of the European baseline series of epicutaneous tests established by the European Environmental and Contact Dermatitis Research Group. We also considered substances that the patient was occupationally exposed to.

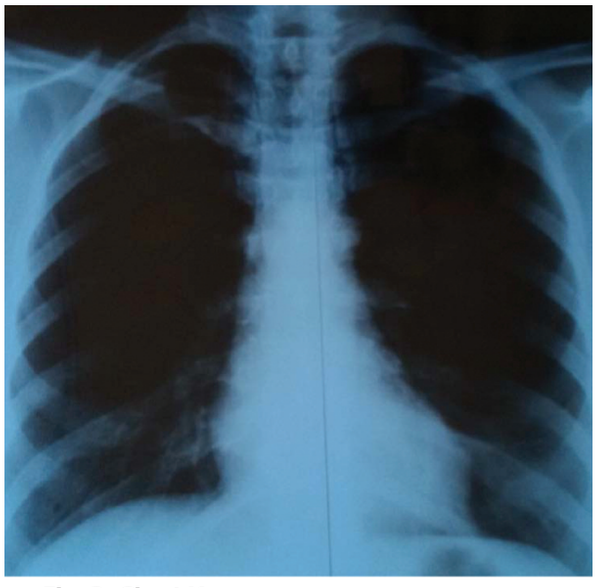

Fig. 2. ***Chest X-ray***

The X-ray indicated basal peribronchial fibrosis near the heart, hilar congestion, and clear costodiaphragmatic recesses. **(Fig. 2.)**

Therapeutic regimen

We started a complex antiallergic treatment to alleviate respiratory and skin-allergic symptoms – patient was started on a parenteral corticosteroid, oral H1- and H2-blockers, and a combined medication in the form of aerosol (ICS +LABA).

Outcome of hospitalization

After six days of hospital stay, patient was discharged with clinical and physical improvement, and in a good general status. Hives remitted completely after the treatment, and bronchial asthma was completely controlled. We gave written recommendations to the patient and her general practitioner regarding the need for a follow-up and clinical and functional control of her asthma. A medication regimen was prescribed to complete the treatment for urticaria. Treatment for asthma control was also prescribed. A recommendation was made for monitoring patient's condition by specialists in allergology and occupational diseases.

Discussion

It is well-known that various types of wheat, seeds, plants, fodder and other foodstuffs produced by the agricultural industry can cause allergic reactions and diseases [5, 22, 23, 26, 31]. Wheat flour is one such example, and our patient was directly exposed to it at the workplace [22, 25, 26, 29, 31].

Some types of plant protein may act as complete antigens and sensitize occupationally exposed people, thereby inducing immediate hypersensitivity reactions [22]. Following inhalation of dust from decaying hay, thermophilic actinomycetes may cause immune-allergic inflammation of lung parenchyma. This mechanism is the basis for the most common and well-studied type of hypersensitivity pneumonitis, „Farmer's lung" [25, 26, 28]. Farms exposed to fermented sugar cane may develop another form of exogenous allergic alveolitis, that is „Bagassosis" [26, 31].

„Byssinosis", whose pathogenesis is pharmacodynamical, may develop in people occupationally exposed to dust from cotton, linen, jute, hemp. Non-immune release of bronchoconstriction substances and mediators, such as histamine and 5-hydroxytriptamine, forms the basis of bronchial obstruction syndrome in those patients [26, 31]. Patch test is a key method for diagnosing contact allergic dermatitis. In is known that the latter is the most common skin disease with occupational etiology [25, 26, 28]. Epicutaneous testing is used to establish contact type sensitization (cell-mediated or type IV hypersensitivity, according to the classification of Coombs and Gell). Positive reaction to wheat flour confirms both the allergic mechanism underlying urticaria (dermatitis) and its occupational etiology. Given the elevated levels of IgE, atopic dermatitis characterized by different etiology, pathogenesis and clinical presentation may be discussed in the outline of a broad differential diagnosis. However, high IgE, taken together with the sensitization to various allergens, confirms the allergic nature of patient's asthma.

Based on the results from different tests, we found evidence of five concomitant allergic diseases: asthma with a predominant allergic component (atopic asthma), allergic rhinitis, allergic conjunctivitis, urticaria/dermatitis, angioedema. Allergic mechanisms underlying asthma, rhinitis, conjunctivitis, and urticaria are indicated by the results of the interview, clinical presentation, and allergic status, and are confirmed by the results of other diagnostic procedures.

Table 6 shows the criteria that confirm the occupational etiology of patients' allergic diseases. Those meet with the occupational disease criteria established as a legal prerequisite for confirming the occupational nature of that disease [22, 26, 27, 29, 31].

Table 6. ***Criteria for establishing occupational etiology of allergic diseases***

Criteria	Clinical presentation (diagnoses, syndromes)	Gratis period	Prerequisites for recognition of occupational disease
Etiologic factors Allergens found in foodstuffs (protein), toxic substances originating from plants treated with pesticides during their cultivation, storage, and transportation, inhaled dust from grain and provender. **Occupational hygienic factors** Job title and tasks (in this case – a direct contact with wheat flour).	1) Bronchial asthma 2) Allergic rhinitis 3) Urticaria 4) Angioedema 5) Allergic conjunctivitis	1) In those with atopy – not needed 2) In the other types – 1 to 10 years depending on the type and duration of exposure	1) Confirmed contact at work 2) Paraclinical tests: - Chest X-ray - Pulmonary Function Test - CT scan - total and antigen-specific IgE - provoking tests and tests of shock organ - Elimination, exposure and collection tests 3) Otorhinolaryngologica and ophthalmologic examination

In conclusion, what distinguishes the presented case is:

- The occurrence of several allergic diseases in the same individual;
- The combination of different etiological factors and pathogenetic mechanisms;
- The role of job title as a predictor and a factor leading to manifestation of allergic symptoms.

Conclusions

The combination of several allergic diseases, diverse in clinical presentation, in bread production workers is an opportunity that has to be considered in expert evaluation. Allergic diseases, diverse in their etiology and pathogenesis, can occur concomitantly in the same patient. Clinical presentation and etiology of allergic diseases can be multifaceted. Having adequate knowledge of occupational risk factors and mechanisms of allergic diseases is a prerequisite for employing a personalized diagnostic and treatment approach in these patients.

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Разтворим трансферинов рецептор и феритинов индекс при някои анемични състояния в клиничната практика

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Soluble Transferrin Receptor (sTfR) and sTfR/log Ferritin Index in Several Anemia Conditions in Clinical Practice

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РЕЗЮМЕ:

Разтворимият трансферинов рецептор (sTfR) и индексът му с феритина (sTfR/logFerr) се предлагат в диагностичния подход за железен дефицит (ЖД) и желязо-дефицитна анемия (ЖДА), особено при усложнена анемия, т.к. не са повлияват при възпаление. sTfR е тясно свързан с еритропоетичната активност на костния мозък и промени в нея, може да повлияят информативната стойност на параметъра. Цел:Да се изследва информативната стойност на sTfR и sTfR/logFerr индекс в клинични условия чрез сравняването им при някоианемични, с диферециалнодиагностичен интерес. Материали и методи: Общо 97 пациенти на възраст над 18 години са включени в групи: ЖДА (n=25), анемия при хронично заболяване или възпаление (АХЗ, n=25), хетерозиготна бета-таласемия (β-ТТ, n = 15), хронична бъбречна недостатъчност на хемодиализа (ХД, n=14) и терапия с еритропоетин стимули-

ABSTRACT

Soluble transferrin receptor(sTfR)and its ferritin index (sTfR/logFerr) aresuggested in the diagnostic approach of iron deficiency(ID) and iron-deficiency anemia (IDA), especially in complicated anemia, as they arenot influenced by inflammation.sTfR is closely related to erythropoietic activity of the bone marrow, which may influence the informative value of the parameter. Aim:The aim of the present study was to investigate the informative value of sTfR and sTfR/logFerr index in clinical settings by comparing them in several anemia conditions of differential interest.Materials and methods:A total of 97 patients over 18 years of age were included in groups: IDA (n=25), anemia of chronic disease or inflammation (ACD) (n=25), β-thalassemia trait (β-TT) (n=15), chronic kidney disease on dialysis (HD) and erythropoietin-stimulating agents (ESA) therapy(n=14), and patients without anemia (n=18) as a control group (CG).Complete blood count,iron status

ращи препарати (ESA), и пациенти без анемия (n=18). Изследвани сапълна кръвна картина, показатели на железен статус, sTfR и феритинов индекс. Резултати:Медианите на sTfR в групите ЖДА (4,41 mg/l) и β-ТТ (2,62 mg/l) са над референтния интервал поради ЖД и лека еритроидна хиперплазия и се различават значително (p<0,01), което е свързано с тежестта на ЖД и анемията. Феритиновият индекс в групата ЖДА (медиана 5.6) е над 2 - предсказваща стойност за абсолютенЖД, и значително по-нискък - под 2 в групата на β-ТТ (медиана 1.26) (p <0.01). Резултатите от sTfR и индекса са сходни при групите с АХЗ (медиани 1,76mg/l и 0,70) и на ХДс ESA лечение (медиана 1,60 mg/l и 0,62). В сравнение с КГ (1,06mg/l), медианите на sTfR са леко повишениприАХЗ (p <0,05) и при ХД група (p<0,01), но са в референтния интервал,като е възможно влияние на функционален ЖД и ESA терапия. Резултатите от sTfR/logFerr в АХЗ, ХД и КГ (средно 0,61) са с подобни стойности и под 1, която изключва ЖДА. Заключение: Комбинираният подход на sTfR и феритин като индекс допринася за информативната стойност на лабораторния параметър, като спомага за преодоляването на недостатъците при използването на sTfR като единичен тест за железен статус при анемия, а интерпретацията на резултатите е нужно да е в контекста на клиничните състояния.

Ключови думи: разтворим трансферинов рецептор, феритинов индекс, анемия.

parameters, soluble transferrin receptor (sTfR) andsTfR/logFerr indexwere determined.Results:The sTfR mediansin IDA (4,41mg/l) and β-TT (2,62mg/l) groups were over the reference interval because of ID and mild erythroid hyperplasia, and differed significantly (p<0.01), which is connected to ID and anemia severity.The ferritin index in IDA group (median 5.6) was above 2 - the suggestive value of absolute ID, andconsiderably lower - less than 2 in β-TT (median 1.26) group (p<0.01).Results of sTfR and ferritin index were similar in ACD (median 1,76mg/l and 0,70) and HD group with ESA treatment (median 1,60mg/l and 0,62). When compared with CG(median 1,06mg/l),sTfRmedians were higher both in ACD (p<0.05) and HD group (p<0.01), with possible effect of functional ID and ESA, but still within reference interval. sTfR/logFerr index results in ACD, HD and CG(median 0,61) were of similar values and below 1 which excludes IDA.Conclusion:The combine approach of sTfR and ferritin as index contributes to informative value of the laboratory parameter and appear to help overcoming the drawbacks to the use of sTfRas a single test for iron status in anemia. The results should be interpreted in the context of clinical condition to improve their informative value in medical practice.

Key words: soluble transferrin receptor – sTfR/log Ferritin index - anemia.

Introduction

Soluble transferrin receptor (sTfR) is a truncated form in serum of membrane transferrin receptor 1 – functional receptor involved in iron delivery to cells.This soluble form derivesmainly from erythroblasts and reticulocytes and its concentration in serum is proportional to the amount of cellular receptor and erythroid precursors in the bone marrow [1]. Transferrin receptor synthesis is increased in case of iron deficiency, leading to corresponding increase in sTfR concentration. The serum level of sTfR is closely related to cellular iron demands and erythroid proliferation rate[1,10]. The sTfR measurement is biomarker of tissue ID and bone marrow demand for iron, not directly or significantly influenced by infection or inflammation, and issuggested in the diagnostic approach of ID and IDA especially in patients with inflammatory and chronic diseases, whenserum ferritin is elevated and informative [2,7,9].The ratio between sTfR and the logarithm of serum ferritin (sTfR/log10ferritin), named sTfR-ferririn index, has been suggested as a biomarker that fully assesses body iron stores, with higher sensitivity and specificity in comparison to the use of sTfR or ferritin alone, for the detection

of ID in anemia in inflammatory conditions [7,10].

The diagnosis of IDA is important for clinicians not only as a symptom for further diagnostic investigation to discover the underlying disease. IDA requires iron replacement therapyeven in complicated conditions like inflammation and chronic disease, and when correctly distinguished may prevent unnecessary iron treatment with further adverse events[3,13]. Soluble transferrin receptor and its ferritin index are suggested in ID diagnostic approach in such situations [8,11].Soluble transferrin receptor concentrations can be raised also in conditions with stimulated erythropoiesis - hemolytic anemias like beta-thalassemia and therapy with erythropoietin-stimulating agents (ESA) [1,9,10], which may influence the interpretationofsTfR results.

AIM

Soluble transferrin receptor is a new routine parameter in our laboratory and available for use in complicated anemia clinical situations. The purpose of the present study was to investigate the informative value of sTfR and sTfR-ferririn index in clinical settings by comparing them in several anemia conditions.

Materials and methods

Patients:The study included 97 consecutive patients over 18 years of age with iron-deficiency anemia (IDA; n=25), anemia of chronic disease or inflammation (ACD; n=25), β-thalassemia trait (β-TT; n=15), chronic kidney disease on dialysis (HD; n=14), and patients without anemia (n=18). They were consulted or diagnosed and treated in Hematology Clinic and Hemodialysis wardof Military Medical Academy, Sofia. Prior written informed consent of patients was obtained for blood sampling as a part of the common diagnostic work-up process. The median age of the patients was 50.4 years (20 to 91), of which 44 were men (45.4%) and 53 were women (54.6%). According to WHO guidelines anemia was diagnosed at a hemoglobin concentration less than 130g/l for men and less than 120g/l for women[14]. Patients were selected for four study groups of interest based on the medical history, physical examination,routine blood tests and underlying disease. The clinic diagnosis of IDA patients were gastrointestinal diseases (13), gynecological conditions and diseases (12), and of ACD patients - pulmonary diseases and inflammation (3), rheumatic diseases (5), autoimmune diseases (2) and hematological diseases and solid tumors (6), without bone marrow involvement, renal diseases and infections (6), complicated diabetes (3).All patients on chronic HD were being treated with a standard 4-hour bicarbonate dialysis three times per week, and were given ESA(at a dose of2000 to 4000 IU at each dialysis) and regular intravenous iron therapy.The group without anemia,which was used as a control group (CG), consisted of 18 patientswho exhibited no abnormal hematologic findings in complete blood count, and serum ferritin and TIBC were within reference ranges.Demographic data of included patients by groups are presented in **Table 1.**

Laboratory analysis: Routine laboratory testing included complete blood count and iron profile. All laboratory investigations were carried out in the Central clinical laboratory of Military Medical Academy, Sofia. The laboratory participates in external quality assessment and has relevant certificates. Blood samples were taken in a fasting state in the morning before any iron supplementation treatment or dialysis,and the laboratory analysis was carried out within two hours.

Hematological parameters such as hemoglobin (Hb),mean cell volume (MCV), mean cell hemoglobin (MCH), reticulocyte count, were measured as complete blood count on the ADVIA 2120 and Sysmex-XN 2000 automatic hematologic analyzers. The corrected reticulocyte index (CRI) was calculated as: CRI = Ret(%)xHct/0,45.

Table 1. ***Demographic data of patients in different groups***

Parameter	IDA (n=25)	β-TT (n=15)	ACD (n=25)	HD (n=14)	CG (n=18)	All patients (n=97)
Age (years) *Mean (Range)*	42.8 (23-78)	48.3 (20-69)	61.9 (32-91)	54.8 (33-83)	43.3 (22-85)	50.4 (20-91)
Sex (n) *male/female*	7/18	6/9	12/13	11/3	8/10	44/53

Biochemicalmarkers of iron status - serum iron (Fe; m.12-32μmol/l, f.10-32μmol/l), transferrin (Trf; 2.0-3.6g/l), transferrin saturation (TSAT; 16-46%) and ferritin (Ferr;m.20-250μg/l, f.10-120μg/l), were measured on the Beckman-Coulter Olympus 680 automated biochemical analyzer, as well as C-reactive protein (CRP;0-5mg/l) as a parameter for inflammation.Total iron binding capacity (TIBC; 44.8-80.6μmol/l) was calculated using the follow formula: TIBC (μmol/l)=Trf (g/l) x 25,1.

Serum concentration of sTfR was determined via Beckman-Coulter analyzer Access 2 with Access® sTfR immunoassay system. The sTfR-ferritin index was calculated as: sTfR/logFerr index= sTfR(mg/l)/logFerr(μg/l), where log is a decimal logarithm [7,11].The assay reference interval for sTfR was: 0.9 - 2.1 mg/l.

Statistical analysis: Descriptive data analysis was used for categorical and continuous variables.Shapiro-Wilk test was applied to investigate the normality of the data. Mann-Whitney U test was used to compare two groups.Correlation indices were calculated. Significance of conclusions was determined at $p<0.05$. The statistical analysis was performed with statistical software SPSS-19.

Results

The results of hematology parameters and markers of iron status in the different groups are presented in **Table 2.** Clinical groups were well defined, justified by the results of their laboratory parameters. Anemia showed the lowest hemoglobin concentrations in IDA group and its characteristic in patients with IDA and β-TT was of microcytic, hypochromic anemia, more manifested in thalassemia trait. Patients with IDA showed typical changes of laboratory parameters of iron status for absolute ID, with deplete iron stores and low serum ferritin. Patients with ACD presented with changes for functional ID and iron sequestration with redistribution of iron from the sites of its utilization to storage sites. The hemodialysis group,that also received iron supplementation, and ACD had the highest serum ferritin and CRP levels.

Table 2. ***Hematological and biochemical data of patients and control group***

Parameter	IDA (n=25)	β-TT (n=15)	ACD (n=25)	HD (n=14)	CG (n=18)
Hb (g/l)	86.0 (57.6-114.4)	111.0 (93.0-128.0	105.0 (84.6-120.4)	106.6 (68.5-124.5)	137.0 (127-151.1)
MCV (fl)	74.4 (65.2-82.1)	63.6 (61.7-72.5)	89.0 (78.2-98.1)	94.0 (87.6-101.4)	87.9 (82.1-95.0)
MCH (pg)	21.3 (16.7-25.3)	19.7 (17.5-22.3)	28.8 (24.9-31.2)	30.3 (26.5-32.1)	30.0 (27.0-33.4)
CRI (%)	1.3 (0.5-2.3)	1.6 (1.1-2.3)	1.3 (0.7-2.5)	1.3 (0.6-2.6)	1.4 (0.7-1.8)
Fe (μmol/l)	2.5 (1.0-5.8)	13.9 (8.4-28.2)	6.0 (2.4-12.6)	15.4 (4.2-20.8)	13.4 (6.4-21.9)
TIBC (μmol/l)	90.4 (76.8-126)	67.0 (51.4-80.3)	50.2 (36.6-65.2)	45.1 (36.4-63.5)	66.5 (59.7-80.6)
TSAT (%)	2.7 (0.7-6.0)	24.1 (9.6-42.1)	13.9 (5.4-23.7)	32.6 (9.5-47.6)	22.1 (9.6-34.3)
Ferr (μg/l)	6.0 (3.6-14.2)	152.7 (15.6-255.4)	225.0 (45.8-641.8)	733.0 (109-1137)	70.5 (16.5-282)
CRP (mg/l)	0.8 (0.1-3.4)	0.9 (0.1-13.9)	21.4 (6.3-128.3)	6.3 (0.1-51.1)	1.0 (0.3-4.2)
presented as median and P10-P90					

In 97 patients studied, median sTfR was 1.96mg/l (0.56-11.76mg/l). The concentration of sTfR increases in hyperproliferative erythropoiesis and iron-deficient states. It is known not to be affected directly by inflammation. We investigated the correlation of sTfR with Hb level, ferritin, CRI andCRP in patients. Soluble transferrin receptor showed significant negative correlation with hemoglobin (r=-0.577, p<0.001), moderate with ferritin (r=-0.306, p<0.001), and not direct weak, negative correlation with CRP (rho=-0.289, p<0.005), but no correlation between sTfR and CRI was found.No sex-related differences or age association of sTfRlevels were observed.

The distribution of sTfR and sTfR-ferritin index values by groups is shown in Figure1. Patients with IDA had the highest sTfR with median of 4.41mg/l, and the highest ferritin index with median of 5.6. Subjects without anemia showed the lowest sTfR with median of 1.06mg/l and the lowest ferritin index with median of 0.61.

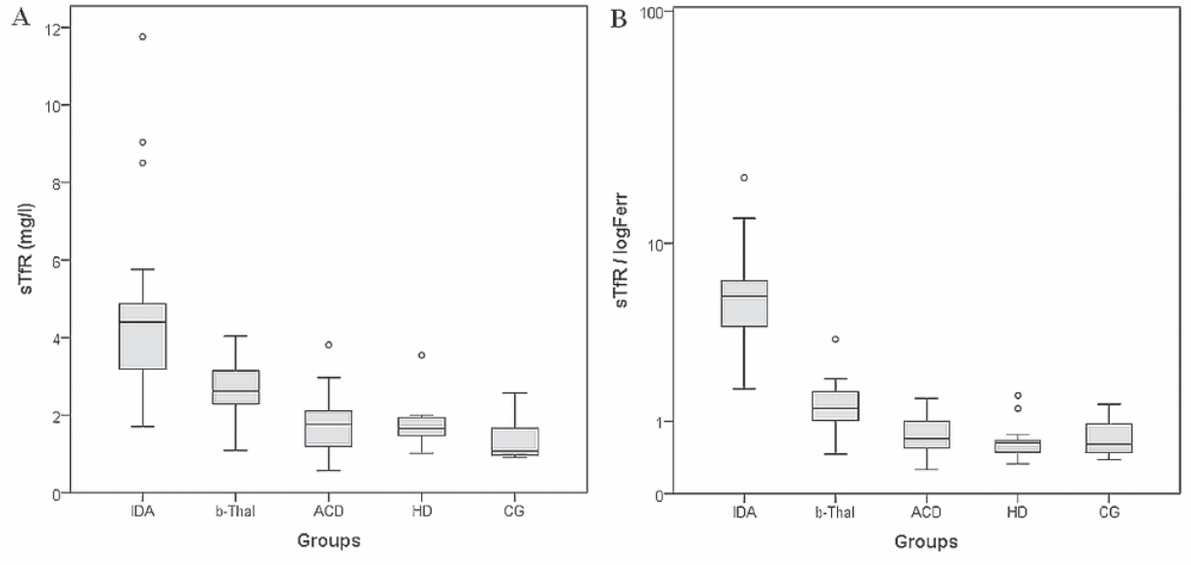

Figure 1. ***(A) Distribution of sTfR values by groups. (B) Distribution of sTfR/logFerr index values by groups in logarithmic scale (presented by 25th, median, and 75th percentiles).***

The results obtained from the sTfRand sTfR-ferritinare presented in **Table 3**. The medians of sTfRserum concentrations in IDA patients and thalassemia trait group wereover the reference interval of the parameter, and the medians of ACD, HD and CG groups were within the assay reference interval.

Table 3. ***Soluble transferrin receptor and sTfR/logFerritin index of patients and control group***

Parameter	IDA (n=25)	β-TT (n=15)	ACD (n=25)	HD (n=14)	CG (n=18)
sTfR(mg/l)	4.41 (2.23-8.72)	2.62 (1.31-3.96)	1.76 (0.82-2.75)	1.60 (1.10-2.76)	1.06 (0.91-1.92)
sTfR/logFerr	5.60 (2.67-12.48)	1.26 (0.73-2.55)	0.70 (0.33-1.32)	0.62 (0.37-1.41)	0.61 (0.39-1.25)
presented as median and P10-P90					

As expected the sTfR levels were statistically higher in IDA patients compared to patients with ACD ($p<0.01$) and CG ($p<0.01$). The same significant differences were found for ferritin index between IDA and ACD ($p<0.01$) and IDA and CG ($p<0.01$). All patients with IDA had higher levels of the index than those with anemia of inflammation and subjects without anemia. Microcytic anemia groups of IDA and thalassemia trait, showed significant difference both in serum level of sTfR ($p<0.01$), and in sTfR-ferritin index ($p<0.01$), which were higher in patients with IDA. When patients with IDA were divided by Hb level - above 90g/l (n=10) and below 90g/l (n=15), and compared separately with β-TT, whose Hb was above 90g/l in all patients, sTfR showed significant difference only for IDA with Hb<90g/l ($p<0.01$), but sTfR-ferritin index was still statistically different ($p<0.01$)in both IDA subgroups. Although receiving ESA therapy patients on hemodialysis had considerably lower sTfR ($p<0.01$) levels and ferritin index ($p<0.01$) compared to IDA group.

Patients with ACD and those on hemodialysis treated with ESA had similar serum concentrations of sTfR and results of sTfR-ferritin index. When compared with patients without anemiasTfRlevels were significantly different both in ACD group ($p<0.05$) and HD group ($p<0.01$), but sTfR-ferritin index results did not differ between the groups.

Hemolytic state of thalassemia trait patients leaded to slightly elevated sTfR and sTfR/logFerr, but statistical differences were found for these two parameters withnonhemolytic states in ACD, HD and CG ($p<0.05$).

Discussion

The comparison of the results found considerabledifferencesin sTfR levels between measured clinical groups. The serum concentrations of sTfR were the highest in IDA group and differed significantly from other groups, although the results showed some degree of overlap (Figure 1A). As expected IDA group showed significantly higher results for sTfR than the control group without anemia and ACD. The relatively mild elevation of sTfR in β-TT differed from CG, which could be connected with mild erythroid hyperplasia usually seen in thalassemia trait. The results of β-TT group were significantly lower compared with IDA group, althoughwith anotable degree of overlap(Figure 1A).Fifteen of 25 patients (60%) with IDA had anemia with Hb<90g/l and all thalassemia trait subjects had Hb>90g/l. This difference in IDA and β-TT groups and the correlation found between sTfR levels with hemoglobinand ferritin concentrations suggest that anemia severity and ID degree are possible factors leading to differences in sTfR concentrations between the groups [5].

Patients from the rest three groups showed medians of sTfR within reference intervals, with the lowest result in subjects without anemia. The groups of ACD and HD had similar values of sTfR that did not differ significantly, even though the latter received ESA conventional therapy. They consist of patients with chronic diseases or inflammation in which ACD is a typical finding, and patients with chronic kidney disease with anemia generally attributed to erythropoietin deficiency, but these clinical conditions share common complex pathogenesis[6,13]. Results of sTfR in both groups were statistically higher than in subjects without anemia, which can be possibly due to functional ID and ESA therapy [3,4,13]. Nevertheless, the differences are small, medians of sTfR were still in assay reference interval (Table 3), and there was substantial degree of overlap of sTfR serum concentrations between groups (Figure 1A), which reflectssTfR was not affected by inflammation or infection. Moreover it showed only negligible weak correlation with CRP.

The sTfR/log Ferrindex results were also the highest in IDA group and significantly different when compared with all other groups, with only small degree of overlap with β-TT, and no overlap with other groups(Figure 1B). The median in IDA group was above 2 - the suggestive value of absolute ID [13]. Ferritin index in β-TT group showed statistically higher results when compared with ACD, HD and CG, for sTfR in the equation. However the median was less than 2 and significantly lower than IDA group.

All patients with IDA had higher sTfR-ferritin indices than patients with ACD, patients on hemodialysis and those without anemia. The later three groups had similar sTfR/logFerr results, which did not differ significantly between groups. Additionally the medians of the ratio were less than 1 - excluding value for IDA in

ACD [13].Determination of sTfR and ferritin, when applied in combination as sTfR/logFerr, reflects the balance between the iron, inflammation and erythropoietic iron requirements of the body.Anemia in chronic kidney diseases is partly related to inflammation activity like anemia in inflammation which causes a rise in serum ferritin. When ferritin index is calculated, it appears to negate sTfR changes for functional ID due to iron restriction in ACD, or ESA therapy effects in CKD [11,12]. Moreover there was no overlap with IDA group for sTfR/logFerr index and therefore the parameter appearsmore informative for ID than sTfR.

Conclusions

In conclusion, the combine approach of sTfR and ferritin as index contributes to informative value of thereceptor andappears to help overcoming the drawbacks to the use of sTfR or serum ferritin as a single test for iron status in anemia. The results should be interpreted in the context of clinical conditions to improve their informative value and guide diagnostic and treatment decisions in clinical practice.

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