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REVIEW

MicroRNAs as Biomarkers and Therapeutic Targets in Heart Failure

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Heart failure still represents a real challenge both in everyday practice and research, due to the complex issues related to its pathogenesis and management. Humoral biomarkers have emerged in the last decades as useful tools in the diagnosis, risk stratification and guiding the treatment of heart failure. These molecules are related to different pathological and adaptive processes, like myocardial injury, neurohormonal activation and cardiac remodeling, their most widespread representatives being the natriuretic peptides (e.g. NT-proBNP). The role of altered gene expression and transcription as the basis of myocardial structural and functional changes in heart failure is largely recognized. MicroRNAs (miRNAs) are non-coding RNAs which have a major role in post-transcriptional gene expression by interfering with messenger RNA molecules. Our short review summarizes the molecular biology of miRNAs and their possible role as biomarkers in the diagnosis and prognosis of heart failure. Furthermore, the therapeutical perspectives conferred by these molecules are also presented.

Keywords: miRNA, biomarkers, heart failure

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Introduction

In heart failure (HF), structural and functional cardiac changes affect the systolic and/or diastolic function of the heart, leading to low cardiac output and increased filling pressures. The resulting global hypoperfusion and pulmonary and systemic congestion trigger the well-known symptoms and signs of HF (dyspnea, fatigue, edema) [1].

The complex pathophysiological processes behind HF are accompanied with altered synthesis (over- or underproduction), or de novo appearance of certain humoral compounds, molecules, called cardiac biomarkers. These substances can be used in the diagnosis and risk stratification of HF. Furthermore, they can be useful also in the follow-up of patients and in the guiding of therapy. Theirs most frequently used representatives are the natriuretic peptides (e.g., N-terminal prohormone brain natriuretic peptide, NT-proBNP) [2].

In many fields of cardiac pathology (e.g., certain arrhythmias, cardiomyopathies, etc.) the investigation and clarification of genetic background (e.g., channellopaties, sarcomere protein gene mutations) is a usual and mandatory part of management. Also, background genetic changes could serve as novel biomarkers with prognostic and therapeutical value. In this regard, microRNAs (miRNAs) a type of non-coding RNAs are promising molecules as biomarkers in the setting of HF. Furthermore, the study of miRNAs could provide a better understanding of molecular mechanisms which play role in the pathogenesis of cardiac morpho-functional changes causing HF [3].

In the followings, we present a short and comprehensive review of the main issues related to circulating miRNAs as biomarkers and future therapeutic targets in HF.

Molecular biology of miRNAs

miRNAs make of the group of non-coding RNAs, together with transfer RNAs, ribosomal RNAs, small nuclear RNAs, small nucleolar RNAs, Piwi-interacting RNAs and long non-coding RNAs. The main role of these molecules is to regulate gene expression by interfering with the functioning and translation of messenger RNAs (mRNAs).

miRNAs are encoded in the genome, and based on their genomic location are classified as intergenic (with own promoters), exonic and intronic (co-expressed or transcribed in reverse orientation of the host genes) types. Transcription by the RNA-polymerase II or III (in fewer cases), results in the primary miRNA, named pri-miRNA (with hairpin shape), which contains 65 nucleotides. In the presence of exportine-5 the pri-miRNA is transported to the cytoplasm, where it is processed by the cytoplasmic RNase III (Dicer family), resulting the mature miRNA (with length of 18 to 24 nucleotides), which integrates in the miRNA-induced silencing complex (miRISC). The miRISC, due to its miRNA content can recognize specific target mRNAs and can determine their translational silencing (gene expression regulation at post-transcriptional level). The miRNA molecule binds to the untranslated parts of the mRNA, triggering its destabilization and degradation, thus, selectively inhibiting protein synthesis [4, 5].

At cellular level, miRNAs influence many processes, like differentiation (fig. 1), proliferation, apoptosis, metabolism and ageing. They could be involved in many pathological pathways, like fibrosis or remodelling, due to their under- or overexpression [6]. A searchable database of known and characterized miRNAs is available at the website www.mirbase.org.

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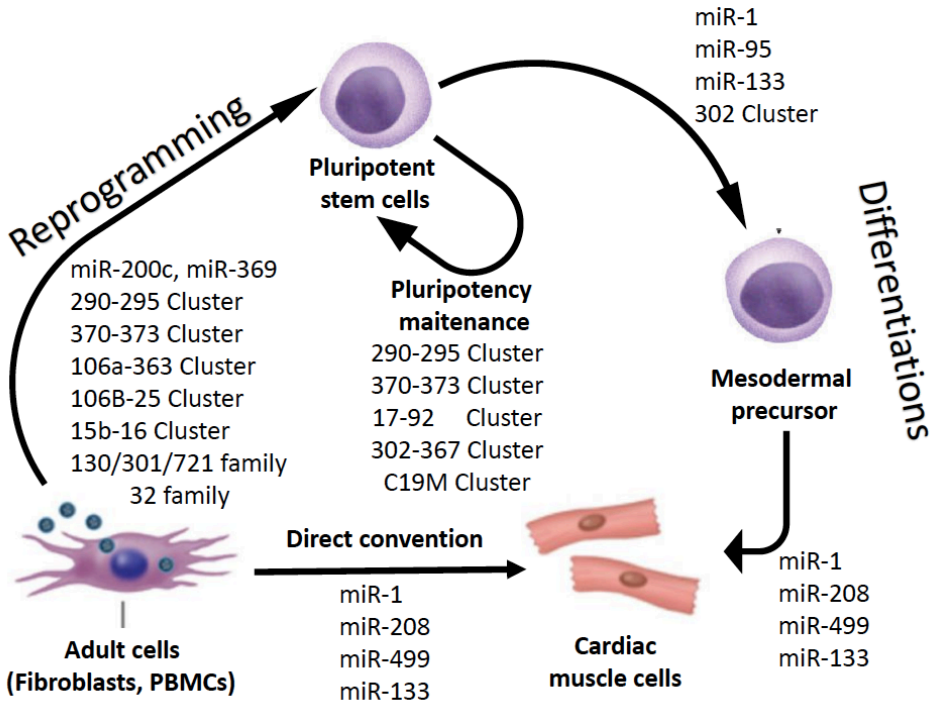

âFig. 1. Representation of the involvement of different miRNAs in cardiac cell differentiation

miRNAs as diagnostic and prognostic biomarkers in heart failure

The changes in the expression of different classes of miRNAs related to various cardiovascular diseases indicate their role in pathogenesis. This confer them utility as potential, novel biomarkers and therapeutical targets. miRNAs could play an important role in the progression of heart diseases even from the beginning, from the initiation of the pathophysiological mechanisms [7, 8]. Table 1 presents the types of miRNAs and their biological actions, with impact on the development of morpho-functional changes leading to HF [9].

Generally, circulating miRNAs could be considered as adequate biomarkers in HF because: a) they can be obtained relatively easily, rapidly and efficiently, and are very stable in the sample; b) certain miRNA profiles are highly specific for certain tissues and pathophysiological processes; c) have the advantage of not suffering post-translational changes [10].

Up- and down-regulation of diverse types of miRNAs, closely related to pathogenesis of HF, could serve as diagnostic and prognostic biomarkers (Table 2) [11, 12]. Many studies demonstrated the presence of a specific circulating miRNA profile in patients with HF. Marfella et al. evaluated a panel of 84 miRNAs previously associated with structural abnormalities of the heart, finding a lower expression of 24 circulating miRNAs in patients with HF. Also, in responders to cardiac resynchronization therapy an increase of 19 types of miRNAs were observed [13]. Apart from some exceptions, several studies mention miR-423-5p as a

Table 2 miRNAs and their potential value as biomarkers in heart failure

Role	MicroRNA	Regulation type in HF	Relation with other biomarkers
Diagnostic	miR-22 miR-92b miR-320a miR-423-5p	Up	+ NT-proBNP
	miRNA-26b-5p miRNA-29a-3p miRNA-30e-5p miRNA-92a-3p	Down	- NT-proBNP
	miR-499 miR-208b	Elevated after acute HF	+ cTnT
	miR-1 miR-21 miR133a miR-208	Time dependent changes after AMI	without correlation
Prognostic	miR-182	Up	superior to NT-proBNP in the prediction of future cardiovascular mortality

"+" = positive correlation, "-" = negative correlation

Table 1 MiRNAs and their biological actions, with (modulating) impact on the development of heart failure

Biological action	MiRNA type
Pro-hypertrophic	155, 199a, 199b, 19a/b, 208a, 21, 21-3p, 212/132, 22, 221, 23a, 27b, 30a, 328, 350
Anti-hypertrophic	1, 101, 133, 145, 150, 185, 223, 26b, 30-3p, 34a, 378, 9, 98
Pro-fibrotic	125b, 21
Anti-fibrotic	7i, 101a, 133, 30, 133a, 24, 26a, 29, 29b
Pro-apoptotic	1, 140, 146b, 15b, 17-5p, 181a, 195, 210, 26a, 30b, 30d, 34a, 497, 539, 92a
Anti-apoptotic	132, 133, 133a, 138, 144, 145, 149, 17, 185, 199a, 20a, 21, 214, 24, 25, 30, 378, 494, 499, 702, 761, 7a/b

useful biomarker associated with HF. In one of the earliest studies in this field, Tijsen et al. identified 6 miRNAs that were elevated in patients with HF, among them miR-423-5p showing a strong association with the clinical diagnosis and the levels of BNP [14]. Various studies have suggested that miRNAs are able to differentiate diverse forms of HF, and could be used as novel biomarkers of HF with preserved ejection fraction [15, 16]. However, miRNAs are not yet recommended by current expert opinions to be used as independent biomarkers in HF, because of the lack of data on large patient populations.

miRNAs as therapeutic targets in heart failure

As we stated above, recent data support, that miRNAs are involved in the onset and development of different pathophysiological pathways playing role in HF. The resulted new paradigm is about their use as potential and innovative therapeutic targets. The main approaches of modulating miRNA activity are the followings: (1) a miRNA mimic is a chemically created double-stranded RNA molecule, which imitates the endogenous miRNA (pre-miRNA) and binds to the complementary mRNA. The specific delivery to cardiac cells involves the use of viral vectors with high affinity to the myocardium [17]; (2) an antagomiR is a synthetic, single strand oligonucleotide that is complementary to a specific mature miRNA, and inhibits its action by binding with it [18]; (3) a miRNA sponge contains several sequences for a particular miRNA, and is capable to reduce the number of active miRNAs [8].

Summarizing the action of these molecules: they are replacing those miRNAs that are under-expressed and are silencing those over-expressed. In this regard, the first experimental data using antagomirs are promising in reverting cardiac fibrosis and hypertrophy [19, 20].

Conclusions

Molecular biology represents a new and potent tool in better understanding and more efficiently treating HF. miRNAs, a class of non-coding RNAs, have a proven role in many pathophysiological processes involved in the development of HF, and are almost ready to be used as diagnostic and prognostic biomarkers, and also, as therapeutic targets in HF, opening the way of a new paradigm in HF management.

Authors' contribution

István Adorján Szabó (Conceptualization; Methodology; Writing – original draft; Writing – review & editing)
Atilla Frigy (Conceptualization; Methodology; Resources; Supervision; Writing – original draft; Writing – review & editing)

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Conflict of interest

None to declare.

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REVIEW

Atrial Fibrillation – An Orchestra of Classic and Modern Risk Factors

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Over the past years, prevention and control of risk factors has begun to play an important role in the management of patients prone to develop atrial fibrillation (AF). A considerable number of risk factors that contribute to the creation of a predisposing substrate for AF has been identified over the years. Although certain AF risk factors such as age, gender, genetic predisposition, or race are unmodifiable, controlling modifiable risk factors may represent an invaluable tool in the management of AF patients. In the recent decades, numerous studies have evaluated the mechanisms linking different risk factors to AF, but the exact degree of atrial remodeling induced by each factor remains unknown. Elucidating these mechanisms is essential for initiating personalized therapies in patients prone to develop AF. The present review aims to provide an overview of the most relevant modifiable risk factors involved in AF occurrence, with a focus on the mechanisms by which these factors lead to AF initiation and perpetuation.

Keywords: atrial fibrillation, epidemiology, mechanisms, remodeling, risk factors

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Introduction

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, can represent both a cause and a consequence of numerous cardiac and non-cardiac diseases. The prevalence of AF is steadily increasing with the aging of the population and the presence of the arrhythmia is associated with a substantial number of risk factors and clinical outcomes [1]. Numerous AF risk factors have been identified, with a variable degree of reversibility (Table I).

Table I. Atrial fibrillation risk factors

Unmodifiable risk factors	Modifiable risk factors	Partially modifiable risk factors
Age	Obesity	Arterial hypertension
Gender	Sedentary lifestyle	Diabetes mellitus
Genetic background	Physical activity	Heart failure
Race	Smoking	Ischemic heart disease
	Alcohol consumption	Chronic kidney disease
	Air pollution	Obstructive sleep apnea
		Chronic obstructive pulmonary disease

Moreover, a two-way relationship appears to exist between AF and many of its risk factors. Factors such as arterial hypertension, aging, heart failure, or ischemic heart disease have long been recognized as major AF risk factors [1]. Another series of factors such as diabetes mellitus, obesity, sedentary lifestyle, obstructive sleep apnea, chronic kidney disease (CKD), and chronic obstructive pulmonary disease (COPD) have been added to this list of AF risk factors more recently [1,2]. Recent studies have also described a relationship between behavioral and environmental factors, including smoking, chronic alcohol consumption, and air pollution, and AF [1]. Atrial fibrillation pathophysiology has been linked to electrical, structural and autonomic abnormalities, and all risk factors involved in AF pathogenesis have been shown to induce one or several of these abnormalities *(Figure 1)*.

The present review aims to provide an overview of the classic and more modern modifiable AF risk factors and to discuss the main mechanisms through which these factors promote the initiation and/or maintenance of non-valvular AF.

Arterial Hypertension

In the Framingham Heart Study cohort, high blood pressure was associated with a 1.8-fold increased risk of developing AF, and, given the increased prevalence of high blood pressure among the study patients, hypertension was responsible for 14% of all AF cases [1]. Particularly, a positive correlation was found between AF and systolic blood pressure [2].

In addition to inducing ventricular hypertrophy and atrial dilation, arterial hypertension has also been shown to cause hypertrophy at the atrial level, which could contribute to the increased risk of AF in this setting [3]. Reduced left atrial function and progressive atrial fibrosis associated with inflammatory infiltrates have also been reported [3]. Overactivation of the renin-angiotensin-aldosterone system (RAAS) has been incriminated as the most relevant mechanism involved in this hypertension-induced atrial proarrhythmic remodeling [4,5]. Increased expression of the angiotensin-converting enzyme and abnormal angiotensin II type 1 and type 2 receptors expression have been reported in this setting [4,5]. Meanwhile, candesartan, an angiotensin II type 1 receptor blocker, was shown to efficiently block angiotensin II-induced collagen synthesis

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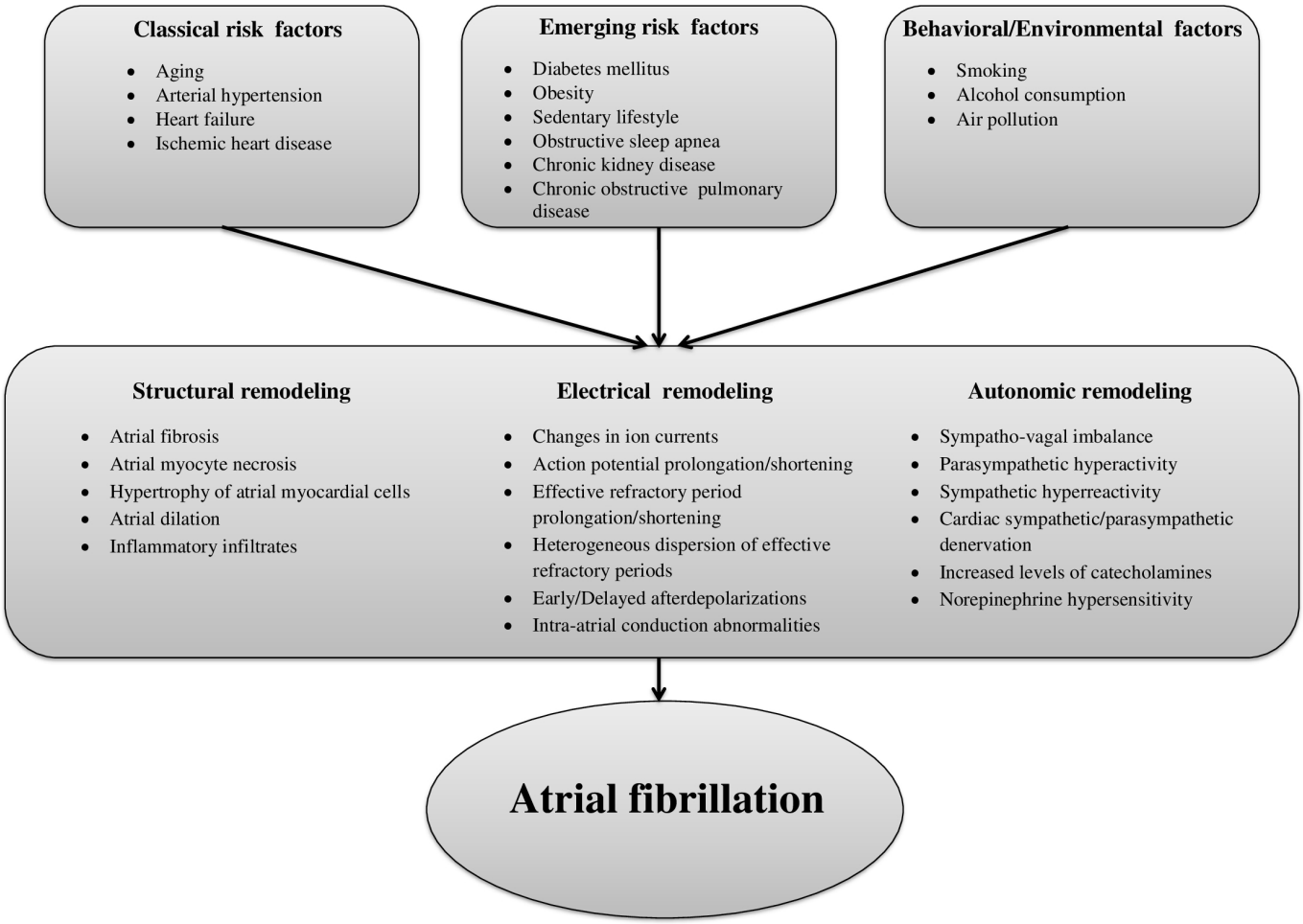

Fig. 1. Schematic representation of the mechanisms linking atrial fibrillation (AF) to its major risk factors. The figure depicts the effects of AF risk factors on the atria, which will ultimately lead to AF initiation and/or maintenance.

and to reduce atrial fibrosis in a hypertensive rat model [6]. Decreased intra-atrial conduction velocity, together with increased heterogeneity and increased duration of atrial effective refractory periods (ERPs), has also been reported in the presence of arterial hypertension [3]. Sympatho-vagal imbalance has been identified more recently as a contributor to AF occurrence in this setting. In spontaneously hypertensive rats, reduced sympathetic tone and relative vagal hyperactivity have been shown to precede and favor AF occurrence [7]. In that study, increased sympathetic tone induced by emotional stress restored the autonomic balance and decreased the number of arrhythmic events, whereas parasympathetic stimulation significantly increased atrial arrhythmic burden and triggered AF [7].

Heart failure

Whereas heart failure is seen as a major risk factor for AF, AF can also be seen as a contributing factor to heart failure [1,8]. In a cohort study, 26% of the patients diagnosed with heart failure developed AF over a mean follow-up of 4.2 years, whereas the incidence of heart failure among AF patients was 33 per 1,000 person-years [8].

In an experimental study in dogs, heart failure was associated with atrial fibrosis, whereas treatment with pirfenidone, an anti-fibrotic agent, attenuated these structural changes and significantly decreased AF susceptibility [9]. Changes in intracellular calcium, characterized by increased calcium transient amplitude and sarcoplasmic reticulum calcium overload, also appear to provide an AF-susceptible substrate in this setting [10]. Prolonged ERP and action potential duration, predisposing to early afterdepolarizations, have been reported in dogs with heart failure [9,10], probably due to decreased activities of the transient outward K^+ current (I_{to}) and of the slow delayed rectifier current (I_{Ks}) [11]. Increased activity of the Na^+/Ca^{2+} exchanger has also been reported in those dogs, favoring delayed afterdepolarizations and AF [11]. However, neurohormonal activation, characterized by increased release of catecholamines and angiotensin II, appears to be the main mechanism linking heart failure to AF [12,13], whereas angiotensin II receptors blockers and angiotensin-converting enzyme inhibitors have been shown to efficiently prevent AF occurrence in this setting [14].

Ischemic heart disease

Ischemic heart disease and AF often coexist in the same patient and can potentiate one another [15]. In the Framingham Heart study cohort, one fourth of men with coronary

heart disease developed chronic AF [16]. The risk to develop transient AF was four times higher in women with than in those without coronary heart disease, although there was no significant association between coronary heart disease and chronic AF [16]. In the same study, a 3-fold and a 9-fold increase in the risk of developing transient AF was also reported in men and women with a previous acute coronary syndrome, respectively [16]. Inflammation, a key player in the pathogenesis of coronary artery disease, has been shown to promote AF *via* structural, electrical, and/or autonomic remodeling [15]. In a study on rabbit isolated left atria, hypoxia induced electrophysiological changes characterized by increased ERP and decreased conduction velocity, increasing vulnerability to reentry [17]. In dogs, atrial ischemia led to local conduction slowing, favoring AF maintenance [18]. Finally, ischemic heart disease leads to myocardial dysfunction and heart failure, which are independent risk factors for AF [1,8].

Diabetes mellitus

Numerous studies have reported an increased incidence of AF in diabetic patients and a linear relationship has been observed between both the duration of diabetes and HbA1c levels and AF risk [19]. However, the diabetes mellitus-AF relationship is far from clear. Although a large amount of data indicates diabetes mellitus as an independent risk factor for AF, to date, there is no definitive proof that diabetes *per se* is sufficient to ensure AF occurrence [19].

Nevertheless, in female patients, impaired glucose tolerance was associated with increased left ventricular mass [20], a known AF risk factor. The systemic inflammatory syndrome commonly encountered in diabetic patients [19] has also been placed amongst the mechanisms linking diabetes to AF. Increased interleukin-6 and C-reactive protein (CRP) levels have been identified in diabetic patients [19], whereas inflammation is known to precipitate AF. Increased fibrosis was also observed in rats with type II diabetes compared to controls, leading to intra-atrial conduction abnormalities [21]. Electrical remodeling of the atria has been reported in diabetic rats, as well as in patients with abnormal glucose metabolism, which displayed intra-atrial conduction abnormalities and decreased atrial voltage [21,22]. Finally, in diabetic rats, inducibility of sustained AF was associated with heterogeneous cardiac sympathetic denervation and homogenous cardiac parasympathetic denervation, and both sympathetic and parasympathetic stimulation have been shown to increase AF occurrence [23].

Obesity

Numerous population-based studies have associated obesity with an increased risk of AF [2,24]. A 4% increase in the risk of AF was observed for each unit added to the body mass index [24], whereas in patients with paroxysmal or persistent AF, weight loss had a dose-dependent effect on maintaining sinus rhythm and reducing the risk of recurrent AF [25].

In sheep, obesity was associated with left atrial enlargement, atrial fibrosis, and pericardial lipid deposits, contributing to AF initiation and maintenance [26]. Direct release of inflammatory cytokines by the epicardial fat has also been incriminated in AF occurrence in obese patients, *via* pericardiac ganglionated plexuses stimulation, parasympathetic-induced ERP shortening and intra-atrial conduction slowing, and sympathetic-induced increase in calcium transient in the atria and the pulmonary veins [15]. In patients undergoing pulmonary vein isolation, obesity has been associated with shorter ERP [27], although inhomogeneous action potential prolongation has also been reported as a potential AF predisposing factor in obese patients [28].

Obstructive sleep apnea

Extensive evidence has associated sleep apnea with an increased risk of developing AF [29]. In addition, sleep apnea and AF share a number of risk factors such as hypertension, obesity, diabetes mellitus, and coronary artery disease [2]. The Sleep Heart Study, which compared patients without sleep-disordered breathing with patients with obstructive sleep apnea, reported a 4-fold higher prevalence of AF in the latter [29], whereas continuous positive airway pressure treatment decreased AF risk in this setting [2].

Repeated forced inspiration leading to decreased intrathoracic pressure favors atrial filling and increases intra-atrial pressure, leading to atrial enlargement and AF [30]. Hypoxia and hypercapnia produced during repeated apnea episodes activate the chemoreceptor reflex, induce autonomic dysfunction, and increase the blood pressure, thus favoring AF occurrence [31]. Inflammation, as evidenced by increased oxidative stress and elevated CRP levels, also appears to promote atrial remodeling and AF in these patients [32]. In addition, the hypercapnia that occurs during apnea episodes has been associated with a uniform increase in ERP and with slowed intra-atrial conduction, mediated by chemoreceptor-induced sympathetic activation [33], while application of a negative pressure during tracheal occlusion, associated with vagal activation, induced a significant decrease in ERP [34].

Sedentary lifestyle and physical activity

There is a nonlinear relationship between physical activity and AF; both sedentary lifestyle and intense physical activity have been associated with increased AF risk [35,36]. In a retrospective cohort study assessing the impact of cardiopulmonary fitness on AF risk, a 7% decrease in AF risk was observed for each metabolic equivalent added during treadmill testing [35]. At the opposite pole, a 5-fold higher risk of AF has been reported in athletes, compared to the general population [36].

The association between reduced physical activity and AF risk factors including diabetes, obesity, and hypertension, is probably one of the main mechanisms by which sedentary lifestyle increases AF risk [37]. Adiposity-asso-

ciated inflammation has been observed in patients with physical inactivity, further contributing to atrial proarrhythmic remodeling [38]. The increased sympathetic tone generally present in these people could also contribute to AF by promoting early and/or delayed afterdepolarizations [39].

Meanwhile, elevated levels of fibrosis biomarkers have been reported in athletes [40] and atrial fibrosis has been highlighted by histological examination in physically trained rats [41]. Atrial dilation, induced by intense physical training as an adaptation to the increased cardiac output, also contributes to AF susceptibility in this setting [41]. Finally, autonomic imbalance has also been incriminated in AF occurrence in trained athletes. Parasympathetic-induced bradycardia, commonly seen in athletes, has been associated with ERP shortening, increased likelihood of reentry, and higher risk of AF occurrence [42].

Chronic kidney disease

Chronic kidney disease is recognized as a strong predictor of cardiovascular events, including AF [43]. The association between CKD and new-onset AF has been reported in several population-based studies and an increased risk of developing AF has been seen in patients with glomerular filtration rate below 60 ml/min/1.73 m^2 [43].

In patients with CKD, the primary role in atrial proarrhythmic remodeling has been attributed to RAAS activation [14]. Low-grade inflammation and increased oxidative stress, commonly seen in patients with CKD, may also play an important role in this regard [44]. Meanwhile, in a model of renal failure in rats, administration of antioxidant agents significantly reduced AF inducibility [44]. Increased levels of catecholamines and enhanced norepinephrine hypersensitivity, typically seen in CKD patients, may also contribute to increased AF susceptibility in this setting [45].

Chronic obstructive pulmonary disease

Numerous studies have linked the presence and the exacerbations of COPD with an increased risk of AF [46,47], whereas forced expiratory volume was negatively correlated with AF occurrence [48]. The role of hypoxia as a possible mechanism for AF promotion in the setting of COPD remains controversial. While some studies have failed to demonstrate a direct effect of hypoxia on the electrophysiological properties of atria [33], others described a hypoxemia-induced inhomogeneous conduction of premature wavefronts, shortened wavelength [18], and sympathetic overactivation [31]. Meanwhile, a consensus seems to have been reached regarding the impact of hypercapnia on the electrophysiological properties of the atria. Hypercapnia-induced atrial conduction slowing and ERP prolongation appear to create a substrate for AF, even after carbon dioxide returns to normal values [33]. In COPD patients, AF occurrence has been linked to prolonged atrial depolarization and electromechanical delay [49]. Increased oxidative stress and systemic inflammation have also been observed in COPD patients [50]. Increased right atrial volume secondary to right ventricular systolic dysfunction and pulmonary arterial hypertension could further increase the AF risk in this population [46].

Smoking

Both current and former smokers seem to have increased risk of developing AF and a dose-response relationship appears to characterize this association [51]. Indirectly, smoking predisposes to myocardial infarction, heart failure, and COPD, all of which are independent risk factors for AF. However, the smoking-AF association appears to extend far beyond these smoking-related conditions. One of the main mechanisms by which tobacco smoking induces AF appears to be myocardial ischaemia, mainly due to decreased blood oxygen carrying capacity, coronary vasoconstriction, and accelerated atherosclerosis [52]. Interstitial fibrosis that occurs in these patients has been attributed to increased transforming growth factor β (TGF-β) and type II TGF-β receptors levels [53]. Nicotine-induced autonomic dysfunction, characterized by down-regulation of *beta*-adrenergic receptors and increased release of catecholamines, has also been incriminated in AF development [54]. Although the effect of nicotine on atrial ion channels has not been studied, at the ventricular level, nicotine-induced blockade of the inward rectifier potassium channels could promote ectopic and triggered activity and induce arrhythmias [55].

Alcohol consumption

The association between alcohol consumption and AF appears to be dose- and gender-dependent [56]. Whereas low alcohol intake does not seem to be associated with AF, moderate alcohol consumption has only been associated with AF in males, and a gender-independent association has been observed between high doses of alcohol and AF occurrence [56]. An association between excessive acute alcohol intake and cardiac arrhythmias, known as the "Holiday heart syndrome", has also been described [56]. One of the mechanisms explaining the alcohol intake-AF association relies on the proarrhythmic effect of acetaldehyde, the primary metabolite of alcohol, on the Purkinje fibers [57]. To date, the effect of alcohol consumption on atrial structural remodeling has not been adequately studied. Qiao et al. recently reported an association between alcohol consumption and the presence of low-voltage areas, speculating a relationship between alcohol consumption and atrial fibrosis [58]. Meanwhile, alcohol-induced electrical remodeling has been studied more extensively. Prolonged (120-h) exposure of rabbits to high intravenous alcohol infusion resulted in a significant decrease in $I_{Ca\text{-}L}$ and sodium current (I_{Na}) density [59]. Decreased ERP and prolonged intra-atrial conduction have also been reported following alcohol ingestion in patients undergoing electrophysiological study [60]. Changes in the duration of the P

wave and of the PR interval further support proarrhythmic intra-atrial conduction prolongation following alcohol consumption [61]. Finally, autonomic dysfunction may also contribute to alcohol intake-related AF. In healthy individuals, acute alcohol ingestion has been associated with a decrease in short-term heart rate variability (HRV) and with an increase in low-to-high-frequency (LF/HF) HRV components ratio, suggesting that sympathetic overactivity may be involved in binge drinking-related AF [62].

Air pollution

Epidemiological evidence, mostly derived from studies in patients with implanted cardioverter defibrillators, associates air pollution with an elevated risk of AF [63], mainly *via* pollution-induced inflammation [64]. In addition, increased levels of pollutants, particularly carbon monoxide and carbon black, have been associated with HRV reduction [65]. Carbonaceous particles have also been shown to increase the LF/HF ratio, demonstrating an increased sympathetic activity in this setting [65]. Increased complexity of the P wave and PR prolongation as a result of acute exposure to particulate matter < 2.5 μm in aerodynamic diameter have also been linked to increased AF vulnerability, although the exact mechanisms by which these changes occur remain to date unknown [66]. Finally, air pollutants can also favor AF occurrence by inducing COPD and heart failure exacerbations and/or by aggravating coronary artery disease [63].

Clinical Implications

Over the time, clinical and experimental studies have identified a variety of AF risk factors and provided insights into their mechanistic links to AF genesis. Elucidating the mechanisms by which various risk factors lead to AF has an indisputable role in identifying new prevention methods. While AF risk factors such as age are unmodifiable (Table I), correction of the numerous modifiable AF risk factors could considerably reduce the AF burden. Indeed, studies have shown that lifestyle changes and/or treatment of clinical conditions commonly associated with AF lead to a significant decrease in the risk of new-onset AF [67]. Although numerous AF risk factors have been identified, clinical risk scores have a limited capacity to predict AF, highlighting once more the complexity of this arrhythmia. Patients often display multiple AF risk factors, and AF mechanisms are probably different from one patient to another. All this suggests that patients at risk of AF probably require a personalized approach and that risk factors management requires an integrated multidisciplinary strategy.

Conclusion

In their vast majority, AF risk factors play an important role in inducing proarrhythmic atrial structural, electrical, and/or autonomic remodeling, but they also often contribute to the development of other risk factors, which will independently contribute, at their turn, to AF initiation and maintenance. Elucidating the mechanisms by which these factors contribute to AF initiation and perpetuation is expected to provide a basis for new antiarrhythmic strategies. In the meantime, adequate management of modifiable AF risk factors could represent a valuable tool for reducing the AF burden in the general population.

Authors'contribution

Alkora Ioana Balan (Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft)

Alina Scridon (Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – review & editing)

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Conflict of interest

None to declare.

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RESEARCH ARTICLE

The Complication Rates of Oral Anticoagulation Therapy in Deep Venous Thrombosis

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The **objective** of the current study is to evaluate the complication rates (embolic and hemorrhagic events) in deep venous thrombosis (DVT) patients on different types of oral anticoagulation therapy (OAC): direct oral anticoagulant therapy and vitamin K antagonist therapy. **Methods:** A number of 62 DVT patients were included and divided in two groups, depending on the type of oral anticoagulation therapy administered. The first group was composed of patients treated with direct oral anticoagulant treatment (Dabigatran, Rivaroxaban) and the second group was composed of patients treated with vitamin K antagonist (Acenocumarol). General data, including BMI and comorbidities were noted. Embolic and hemorrhagic events were noticed. **Results**: in the first group of patients (DOAC therapy), a number of 34 patients were included (14 of them with BMI higher than 25 kg/m2 and 14 with concomitant atrial fibrillation), while the second group comprised of 28 patients treated with VKA (21 of them with a high BMI and only 3 of them with atrial fibrillation). After a mean period of 36 months of anticoagulant therapy, complications were present in 17 patients, hematuria (8 episodes) and pulmonary embolism (4 cases) were the most frequent, with no difference regarding the treatment applied. **Conclusion:** No statistically significant difference was encountered regarding embolic and hemorrhagic event rates in our deep vein thrombosis patients.

Keywords: deep venous thrombosis, direct oral anticoagulants, Acenocumarol, obesity

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Introduction

Deep venous thrombosis (DVT), along with pulmonary embolism (PE) are parts of venous thromboembolic (VTE) disorder with an incidence of nearly 1.6 per 1000 inhabitants a year, affecting especially the lower limbs [1].

DVT is rare if no risk factors are present; the most important risk factors are a history of DVT or pulmonary embolism (PE), sever infection, heart failure, obesity, oral contraceptive treatment, cancer, surgery. More than half of all cases of DVT present with no clinical symptoms. When present, signs and symptoms include local pain and swelling, superficial venous system dilatation [2].

WELLS or modified WELLS score guide the clinician on diagnosing DVT, with a high negative predictive value [3, 4]. Also, D-dimer test and venous ultrasonography of the lower limb are highly accurate in diagnosis.

A frequent and life-threatening complication of DVT is PE, after embolization of a thrombus into the pulmonary arteries. To prevent this important complication, anticoagulant treatment is recommended. Therapies may vary, from parenteral anticoagulation with low molecular weight heparin (LMWH) or unfractionated heparin (UFH) switching to vitamin K antagonist (VKA - Acenocoumarol) or to the direct oral anticoagulants (DOAC), such as Dabigatran, Rivaroxaban or Apixaban.

The aim of this study is to evaluate the complication rate (embolic and hemorrhagic events) in DVT patients on oral anticoagulation therapy.

The protocol of this study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Targu Mures, Romania, number: 171/28.12.2016 in accordance with standards of 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Methods

A retrospective observational study was performed on 62 adult patients with confirmed deep venous thrombosis diagnosis, treated with OAC.

Patients were admitted between 2013, July, 1 – 2016, June, 30, irrespectively of the time when anticoagulation was started or interrupted by various reasons.

Main exclusion criteria were: cancer patients, history of bleeding, major head trauma.

Other comorbidities included the presence of heart failure, systemic hypertension, atrial fibrillation, diabetes mellitus, chronic kidney disease, documented alcohol abuse.

Patients were divided in two groups: group I: 28 patients receiving DOAC's, group II composed of 34 patients with VKA therapy.

We considered the approved regime for the DOAC: Dabigatran 150 mg twice daily (BID) or Rivaroxaban 20 mg once daily (OD). The anticoagulant treatment was applied as recommended.

In group II, Acenocoumarol dose was given so that the patients were in time therapeutic range.

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All included patients had serum creatinine levels clearance over 30mL/min/1.73m^2 (MDRD formula).

For hemorrhagic events four class classification of bleeding provided by American College of Surgeons' advanced trauma life support (ATLS) was used [5].

Bleeding events occurred during the 36 months of treatment were divided in:

– minor events including hospitalization for haematuria, epistaxis, hemoptysis;
– major events including hospitalization for intracranial bleeding, gastrointestinal (GI) bleeding (superior or inferior), and blood transfusion after hemorrhagic events linked to OAC treatment.

The statistical analysis was performed using Microsoft Office Excel 2010 and EpiInfo software. The chi-square statistical analysis was performed. Continuous variables are reported as mean ± standard deviation (SD) and categorical variables as observed number of patients.

In the period 2013 July, 1 – 2016, June, 30, the electronic registries were consulted and each patient was checked for the occurrence of embolic or hemorrhagic events.

Results

The mean age of the entire group was 64±13 years, with no statistical difference between the 2 groups of patients (Table I).

Body mass index (BMI) difference between the 2 studied groups was statistically significant, 21 patients on VKA therapy were overweight, while only 14 patients on DOAC treatment had a BMI over 25 kg/m^2, p value (chi square Yates corrected) = 0.015, OR = 4.285 (1.434-12.806).

Also, atrial fibrillation was present more often in the first group compared to the VKA regimen group, p value (Fisher exact test) =0.047, OR = 0.269 (0.076-0.953).

The mean period of oral anticoagulant treatment of the 62 patients was 32 months.

At the time of the electronic register check of all patients, a total of 17 (27.43%) patients presented with complications, in some patients, more than one complication occurred. The statistical analysis revealed no significant difference between the treatment applied and the complication rate, p =0.637 (Figure 1).

Out of the 17 patients with one or more complications, 8 (47.1%) of them were in group 1 and 9 (52.9%) were in the second group treated with VKA therapy, with no statistically significant difference between the two groups.

The mean age of the 17 patients, cross tabled with the therapy applied, was similar, with no significant differences (Table II).

Regarding a possible association between the BMI and complication rate, 9 of the 17 patients were overweight, with no significant role of the BMI in complication occurrence, p = 0.955.

Also, the BMI showed no significant role in complication occurrence in the two groups taken separately, depending on the type of OAC therapy applied.

Regarding the associated diseases in the 17 patients with complications, chronic kidney disease occurred most often (Figure 2).

Table I. Group characteristics

Baseline group characteristics	Entire group	Group I -NOAC	Group II- VKA	P value
	(nr. of patients/percentage)			
Number of patients	62 (100%)	34 (54.8%)	28 (45.2%)	
Male gender	33 (51.6%)	15 (45.5%)	18 (54.5%)	0.133
Rural area	33 (51.6%)	16 (48.5%)	17 (51.5%)	0.283
BMI - overweight	35 (61.3%)	14 (40%)	21 (60%)	0.015
Associated Diseases				
Heart failure	46 (74.2%)	25 (54.3%)	21 (45.7%)	1
Arterial Hypertension	44 (70.9%)	24 (54.5%)	20 (45.5%)	1
Diabetes mellitus	10 (16.1%)	4 (40%)	6 (60%)	0.494
Chronic kidney disease	43 (69.4%)	24 (55.8%)	19 (44.2%)	1
Alcohol abuse	0	0	0	NA
Atrial fibrillation	17 (27.4%)	14 (82.4%)	3 (17.6%)	0.047

Discussions

A high body mass index (obesity) was proven to have an important role in thrombosis, due to chronic inflammation and impaired fibrinolysis, more and more obese people presenting DVT and the complications that come with it [6]. In our study, high BMI was not proven to have an important role in the occurrence of embolic or hemorrhagic complications in DVT patients.

Studies show that patients with chronic kidney disease have altered the pharmacokinetics of drugs. [7]. Administered in the therapeutic dose direct anticoagulants do not produce more complications compared to acenocoumarol in patients with chronic kidney disease [8].

In the current study, we wanted to determine the benefit of the prophylactic OAC treatment applied to prevent the mentioned complications, using to different classes of OAC. The classic vitamin K antagonist (Acenocumarol) compared to the new class of OAC (Dabigatran, Rivaroxaban).

The results revealed no difference between the two groups regarding the rate of embolic or hemorrhagic complication.

Table II. Complications occurred in the 2 groups

Complications	Group 1	Group 2		P value
		Dabigatran	Rivaroxaban	
Pulmonary embolism	1	1	2	0.619
Stroke/TIA	2	1	0	0.584
Hematuria	8	3	3	0.472
Epistaxis	0	1	0	1
Haemoptysis	0	0	0	-
GI bleeding	0	1	0	1
Blood transfusion	0	0	2	0.496

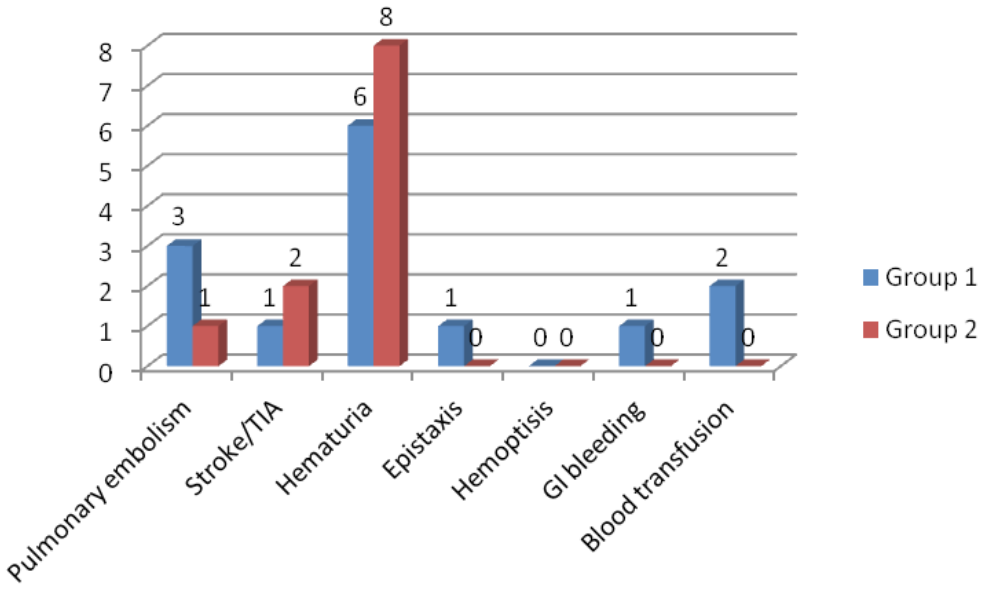

Fig. 1. Complications occurrence rate

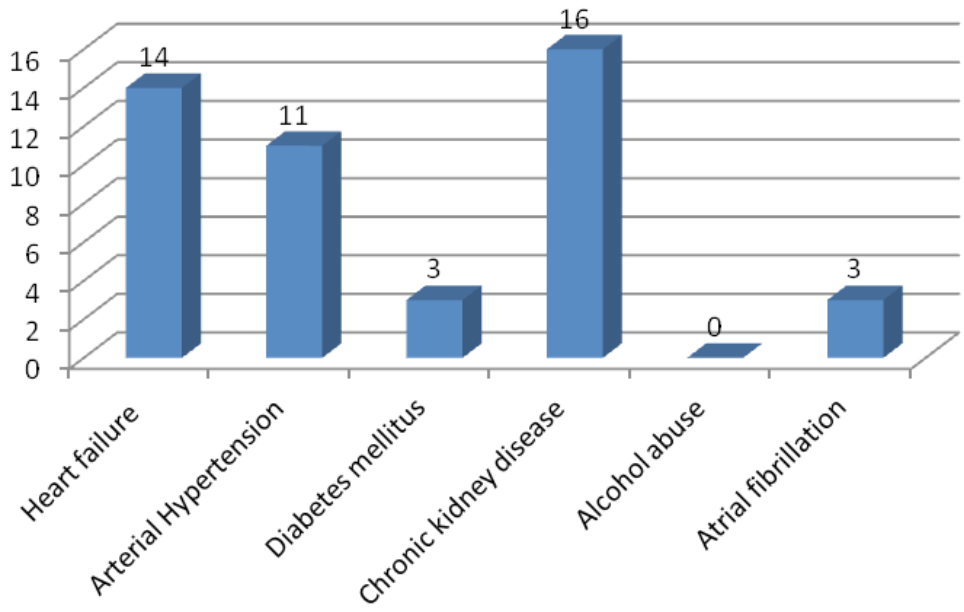

Fig. 2. Associated pathology in patients with complications

Other studies confirm our findings, the DOAC's non inferiority to VKA regarding embolic episodes with lower bleeding risk [9,10].

Conclusions

No statistically significant difference was encountered between the applied class of oral anticoagulation treatment and the embolic or hemorrhagic event rate in our studied deep vein thrombosis patients.

Hematuria and pulmonary embolism were the most frequent complications occurred, with no statistically significant difference between the treatments applied.

High body mass index did no prove to have a significant role in complication occurrence in the two studied groups.

Chronic kidney disease, heart failure, arterial hypertension, atrial fibrillation and diabetes mellitus were the most associated pathologies at baseline, while chronic kidney disease highest encountered at patients with complications.

Authors' contribution

Ionela Silivastru (Cozlea) (Conceptualization; Methodology; Project administration; Writing – original draft)
Arthur-Atilla Keresztesi, (Formal analysis; Software)
Asofie (Keresztesi) Gabriela (Data curation; Investigation; Software)
Daniel Cozlea, (Supervision; Writing – review & editing)
Daniela Ecaterina Dobru (Supervision; Validation)

Abbreviation list

DVT: deep venous thrombosis, OAC: oral anticoagulation, BMI: body mass index, DOAC: direct oral anticoagulation, VKA: vitamin K antagonist, PE: pulmonary

embolism, VTE: venous thromboembolic, LMWH: low molecular weight heparin, UFH: unfractionated heparin, BID: twice daily, OD: once daily, ESC/EHRA: European Society of Cardiology/European Heart Rhytm Association, MDRD: modification of diet in renal disease, ATLS: American College of Surgeons' advanced trauma life support, GI: gastrointestinal, SD: standard deviation, OR: odds ratio.

Conflict of interest

The authors declare there are no conflicts of interest regarding this paper.

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RESEARCH ARTICLE

Markers of Atherosclerosis in Hypertensive Patients with Less Advanced Chronic Kidney Disease

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Objective: Our study aimed to validate the neutrophil-to-lymphocyte ratio (NLR) as a marker for aortic arch calcification in hypertensive patients with less advanced chronic kidney disease (CKD). **Methods**: A number of forty-four hypertensive patients with chronic kidney disease (categories G3a and G3b – 2012 KDIGO nomenclature) were included in the study. Considering the presence of aortic arch calcification (AAC) on chest X-ray, the study population was divided into two groups: 27 patients AAC present and seventeen without aortic arch calcification. Laboratory data were collected for each patient and NLR was computed. Comorbidities were also recorded: stable coronary artery disease, lower extremity arterial disease and hypertensive heart disease. **Results**: A positive correlation between neutrophil-to-lymphocyte ratio and aortic arch calcification in hypertensive CKD patients was identified. Furthermore, advanced age, increased alkaline phosphatase and increased erythrocyte sedimentation rate had a positive association with aortic arch calcification. We found no statistical correlation between neutrophil-to-lymphocyte ratio and other laboratory features in both groups of patients. **Conclusions**: Neutrophil-to-lymphocyte ratio may be viewed as a potential risk factor for vascular calcification in patients with moderate chronic kidney disease; nevertheless, future extensive studies are necessary. In the management of hypertensive patients, general medicine might particularly benefit of this simple, readily available inflammatory marker.

Keywords: neutrophil-to-lymphocyte ratio, atherosclerosis, systemic hypertension, chronic kidney disease, aortic arch calcification

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Introduction

Inflammatory markers tend to increase with age, and additionally, in the elderly population, inflammation is acknowledged as an essential risk factor for morbidity and mortality [1].

Chronic inflammation has been reported as an important prognostic factor for chronic kidney disease (CKD) progression; a high prevalence of it has been long documented in individuals with CKD, particularly in end-stage renal disease patients [2]. Currently, serum markers of inflammation are recognised as potential indicators for early diagnosis, prognosis, and CKD follow-up. C-reactive protein (CRP), interleukin-6 and tumoral necrosis factor-alpha determine injurious effects on the mesangial and endothelial glomerular cells by increasing the production of the extracellular matrix. Moreover, they also lead to degradation and reduction of the stimulated extracellular matrix, hence resulting in renal function decline due to glomerular hypertension, tubulointerstitial fibrosis and renal scarring [3,4]. In haemodialysis patients, CRP remains the most relevant inflammation biomarker, accepted as a validated cardiovascular morbi-mortality prognostic factor [5]. A novel biomarker of inflammation – pentraxin 3 is also associated with CKD progression and may be more sensitive in the prediction of cardiovascular mortality compared to high-sensitive CRP [6-8].

The neutrophil-to-lymphocyte ratio (NLR), recently recognised as an inflammation marker, is an emerging prognostic factor for cancer, cardiovascular diseases [9-13]. No worldwide reference values are validated; European, Asian and Latin-American cohort studies describe different values related to age, gender. In healthy adults, aged under 66 years old, Forget and colab. reported normal NLR values between 0.78 to 3.53 [9].

Inflammation plays an essential role in the pathomechanisms of atherosclerotic disease; additionally, NLR is recognised as a predictor of subclinical and clinical atherosclerotic disease [14,15]. Recent data suggest that NLR may be an indicator for the prevalence and severity of coronary artery disease and also for the extent of aortic arch calcification (AAC) [16,17]. In CKD patients, neutrophil-to-lymphocyte ratio, an inexpensive and convenient available inflammatory marker has also emerged as a prognostic factor for adverse renal events. In the Asian population, NLR is associated with the risk of progression towards end-stage renal disease [18,19].

Vascular calcification, a common finding in CKD patients, is a result of two independent mechanisms. One process, represented by atherosclerotic calcification, is characterized by plaque formation with patchy localisation at the intimal layer followed by calcification induced by osteoblast stimulation; atherosclerotic calcification is related to traditional risk factors for vascular calcification: arterial hypertension, dyslipidemia, smoking, ageing and diabetes mellitus. The second pattern described as arteriosclerosis is characterised by medial artery calcification, as a consequence of non-traditional risk factors for vascular calcification such as hyperphosphatemia, hyperparathyroidism and inappropriate cytokines expression.

Arteriosclerosis is common in patients with chronic kidney disease and diabetes [20]. The extent and type of

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vascular calcifications are acknowledged as mortality predictors due to vascular complications [21, 22].

Aortic arch calcification, identified on conventional posteroanterior chest X-ray, has been recognised as a reliable, independent predictor of cardiovascular events beyond traditional risk factors in CKD patients but also in the general population [23, 24].

Moreover, hypertension alone can promote medial calcification by complex mechanisms which involve increased arterial stiffness. In CKD patients these has paramount implications as hypertension has a frequent occurrence and may lead to premature arterial calcification [25].

Association between NLR and AAC, debated in a large number of papers, emphasized neutrophil-to-lymphocyte ratio as a predictor of arterial calcification extent [26]. On the other hand, the neutrophil-to-lymphocyte ratio proved to be a prognostic factor for vascular calcification in patients with end-stage renal disease as well [22,27].

Both NLR and AAC are associated with arterial stiffness, as neutrophil-to-lymphocyte ratio is independently associated with arterial stiffness in patients with advanced kidney disease, and aortic calcification is associated with arterial stiffness in the general population and hypertensive patients [28-30].

In the present study, we aimed to validate the neutrophil-to-lymphocyte ratio as a marker for aortic arch calcification in hypertensive non end-stage CKD patients.

Methods

Two-hundred eighty electronic patient medical records, enrolled between January 2018 to June 2019, were reviewed and analysed. Main inclusion criteria were represented by the diagnosis of hypertension, chronic kidney disease and availability of posteroanterior chest X-ray in Caucasian patients. Patients with type I and II diabetes mellitus, chronic obstructive pulmonary disease, benign prostatic hyperplasia, acute or chronic infections, cancers or other documented inflammatory conditions were excluded.

We identified 44 hypertensive CKD patients fulfilling inclusion criteria. Chronic kidney disease was diagnosed by the Kidney Disease Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease, based on Glomerular Filtration Rate category criteria [31]. The diagnosis of hypertension was revised accordingly to the 2018 ESC/ESH Arterial Hypertension (Management of) Guidelines using the recorded office blood pressure measures [32]. For all patients, demographic and anthropometric data were collected following a detailed, standardized protocol.

The studied population was divided into two groups based on aortic arch calcification presence on chest X-ray: group 1 consisting of 27 hypertensive CKD patients in conjunction with AAC; group 2 included seventeen hypertensive CKD patients negative for AAC. Calcification of the aortic arch was estimated using the four grades described by Symeonidis et al: grade 0, defined by no sign of calcification; grade 1 – limited proof of calcification in the form of a thin calcification area or small spots; grade 2 – indicative of a more advanced calcification process with one or more extensive calcification areas; grade 3 – aortic knuckle circumferential calcification [33]. All patients enrolled in group 1 had a radiologic proof of aortic arch calcification grade 2 to 3.

The following comorbidities were searched for: evidence of stable coronary artery disease, carotid stenosis, lower extremity arterial disease and hypertensive heart disease.

Presence of stable coronary artery disease (SCAD) was acknowledged in patients who met the 2013 ESC guidelines on the management of stable coronary artery disease, while lower extremity arterial disease (LEAD) was confirmed according to the 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery [34,35]. No history of carotid stenosis was documented in our study population.

For all forty-four patients, complete blood count with full automated differential counts (which included neutrophils and lymphocytes) and serum creatinine values were accessible. Neutrophil-to-lymphocyte ratio (expressed as number) was calculated by dividing the neutrophils count by the lymphocytes count. Estimated glomerular filtration rate (eGFR) was calculated based on the CKD Epidemiology Collaboration (CKD-EPI) formula. Where available, the following data were included in the descriptive and statistical analysis: erythrocyte sedimentation rate (ESR), fibrinogen, iron, total serum calcium, total cholesterol, triglyceride, alkaline phosphatase and uric acid. Due to the lack of availability other routine inflammatory markers (CRP, hs-CRP) were not analysed.

The data processing was carried out using GraphPad Prism 3.1 software (GraphPad Software Inc., San Diego, USA) and Statistical Package for Social Sciences (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, IBM Corp.). Continuous variables are expressed as mean ± standard deviation (SD), and categorical variables are expressed as numbers. In order to assess the statistical significance of differences between the groups, we applied Student's t-test for parametric data; for non-parametric data the Mann-Whitney U test was used. A p-value of <0.05 was considered statistically significant. Correlations between neutrophil-to-lymphocyte ratio and laboratory features were investigated through Spearman's rank correlation coefficient analysis. Regarding comorbidities and NLR association, the variations between the study groups were investigated by the non-parametric Kruskal-Wallis test, and Dunn's multiple comparisons post-test.

This research regarding assessment of atherosclerotic disease in hypertensive patients was approved by the Committee on Medical Ethics, Targu Mures County Emergency Clinical Hospital no. 20874/2019. All procedures performed were in accordance with the ethical standards of

the 1975 Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects, developed by World Medical Association and consequent amendments.

Results

The mean age for group 1 was 72.26±6.567 years old. Out of 27 individuals from group 1, men represented 74.07%. For group 2 the mean age was 65.82±5.399 years old; men represented a total of 52.94% out of 17 individuals.

Baseline characteristics for study participants are illustrated in Table I.

With respect to 2012 KDIGO classification, we included 36 patients in G3a category. Eight cases (18.18% of all patients) were in G3b category. All G3b patients were part of group 1.

Table II depicts comorbidities distribution between the two groups.

Comparative data on age, iron, total cholesterol, triglyceride, ESR, fibrinogen, alkaline phosphatase and uric acid between the groups are displayed in table III. Aortic arch calcification positive patients had significantly higher values for ESR (p=0.034), eGFR (p=0.0007), total cholesterol (p=0.0074) and alkaline phosphatase (p=0.0074) compared to individuals without AAC. Age also proved to be higher in aortic arch calcification positive individuals (statistically strong significant, p=0.0001). A statistically significant difference in other parameters presented in table III was not identified.

Comparative analyses regarding neutrophil-to-lymphocyte ratio distribution in the study population is depicted in figure 1. Aortic arch calcification is associated with increased values of neutrophil-to-lymphocyte ratio when compared with individuals without vascular calcification (p=0.007).

The correlations between NLR and collected laboratory data (alkaline phosphatase, uric acid, fibrinogen, total calcium, total cholesterol, triglycerides) are displayed in table IV and table V. Applying Spearman correlation analysis, we did not report any correlation between neutrophil-to-lymphocyte ratio and laboratory values in aortic arch calcification positive patients or in AAC negative patients.

For analyzing an association between recorded comorbidities and NLR within the two groups, the variations were investigated using Dunn's multiple comparisons post-test, and Kruskal-Wallis test.

We further divided our patients into four subgroups depending on the presence or absence of each comorbid variable. Within the two groups of our study, for each recorded comorbidity we tested the median NLR differences. In the NLR association to SCAD we obtained statistically significant difference in the group 1 - SCAD absence (11 patients) versus group 2 - SCAD presence (10 patients), p=0.0397. A significant p value (p=0.0339) was obtained as a result from the median differences of the NLR in the group 1 without concomitant LEAD (16 patients) versus group 2 without LEAD (fifteen patients). Median NLR values were higher statistically significant (p=0.0375) in aortic arch calcification group 1 patients with concomitant hypertensive heart disease compared to group 2 non hypertensive heart disease (10 patients).

Table I. Baseline characteristics, laboratory features of study group. Reference values of laboratory data are depicted.

Parameters	Patients (N)		Mean ±SD	
	Group 1	Group 2	Group 1	Group 2
Age (years)	27	17	77.26±6.56	65.82±5.39
BMI (kg/m2)	27	17	27.13±4.54	29.93±4.50
ESR (5 – 17 mm/h)	13	6	22.54±16.28	9.66±7.50
Fibrinogen (1.5 – 4.0 g/L)	14	10	7.28±8.82	4.82±0.37
Iron (9.0 – 30.4 µmol/L)	25	16	13.10±4.99	14.20±5.54
eGFR (mL/min/1.73 m2)	27	17	48.04±10.65	59.00±9.17
Total cholesterol (2.8 – 5.2 mmol/L)	26	16	4.38±1.43	4.34±1.16
Triglycerides (0.55 – 1.90 mmol/L)	23	15	1.28±0.44	1.38±1.11
Alkaline phosphatase (100 – 300 U/L)	24	16	258.40±75.80	200.30±50.16
Uric acid (200 – 400 U/L)	27	16	383.60±105.20	352.40±85.75
Neutrophils (1.5 – 7.5x103/µL)	27	17	6.43±1.77	3.86±1.24
Lymphocytes (1.0 – 4.0x103/µL)	27	17	1.71±0.64	1.52±0.38
NLR	27	17	4.33±2.47	2.80±1.29

Abbreviations: BMI – body mass index; eGFR – estimated glomerular filtration rate (CKD-EPI formula); ESR – erythrocyte sedimentation rate; N – number; NLR – neutrophil-to-lymphocyte ratio; SD – standard deviation

Table II. Existing comorbidities of study group (44 patients).

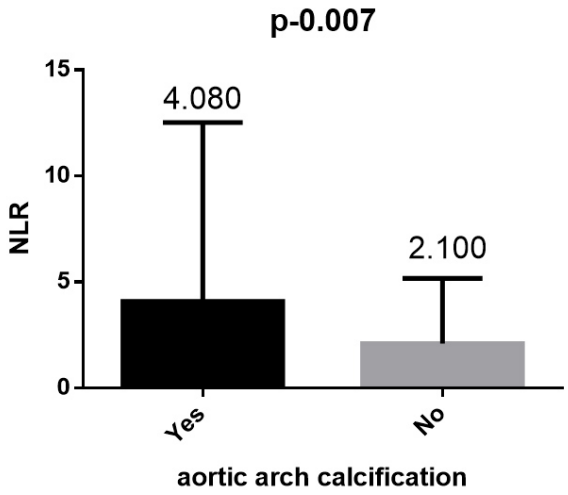
	Presence of SCAD		LEAD diagnosis		Established hypertensive heart disease	
	YES	NO	YES	NO	YES	NO
Group 1 (27 patients)	16 (59.26%)	11 (40.74%)	11 (40.74%)	16 (59.26%)	10 (37.03%)	17 (62.97%)
Group 2 (17 patients)	1 (58.82%)	7 (41.18%)	2 (11.76%)	15 (88.24%)	5 (29.41%)	12 (70.59%)

Abbreviations: LEAD – lower extremity arterial disease; SCAD – stable coronary artery disease. Values are expressed in numbers and percentages.

Table III. Comparison between presence of aortic arch calcification and baseline characteristics of the study groups (Mann–Whitney U test)

Parameters	AAC	N	Median	P value
Age (years)	Group 1	27	77.00	0.0001
	Group 2	17	66.00	
BMI (kg/m2)	Group 1	27	26.00	0.0655
	Group 2	17	29.80	
ESR (mm/h)	Group 1	13	20.00	0.034
	Group 2	6	8.50	
Fibrinogen (g/L)	Group 1	14	4.20	0.612
	Group 2	10	3.65	
Iron (µmol/L)	Group 1	25	13.50	0.5562
	Group 2	16	14.40	
eGFR (mL/min/1.73 m2)	Group 1	27	50.00	0.0007
	Group 2	17	56.00	
Total cholesterol (mmol/L)	Group 1	26	3.74	0.0074
	Group 2	16	4.15	
Triglyceride (mmol/L)	Group 1	23	1.21	0.1554
	Group 2	15	0.95	
Alkaline phosphatase (U/L)	Group 1	24	238.50	0.0074
	Group 2	16	190.50	
Uric acid (U/L)	Group 1	27	381.00	0.3341
	Group 2	16	260.50	

Abbreviations: AAC – aortic arch calcification; BMI – body mass index; ESR – erythrocyte sedimentation rate; eGFR – estimated glomerular filtration rate (CKD-EPI formula); N – number of patients



* Mann–Whitney U test; NLR – neutrophil-to-lymphocyte ratio.

Fig. 1. Comparative analysis on neutrophil-to-lymphocyte ratio between the two groups

Discussion

Chronic kidney disease is currently a significant global health problem, since even with elimination of the generating factor it remains a progressive disease. Initial nephron loss induces adaptive mechanism characterised by hypertrophy and hyperfiltration in the remaining healthy nephrons with subsequent development of glomerular sclerosis characterised by proteinuria and hypertension [36, 37].

Table IV. Correlations between neutrophil-to-lymphocyte ratio and alkaline phosphatase, uric acid in the study group

Parameters	Spearman' rho	NLR		Alkaline phosphatase		Uric acid	
		Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
NLR	Correlation coefficient	1.000	1.000	0.340	-0.135	0.078	0.124
	Sig. (2-tailed)	0	0	0.104	0.619	0.697	0.648
	N	27	17	24	16	27	16
Alkaline phosphatase	Correlation coefficient	0.340	-0.135	1.000	1.000	0.239	-0.195
	Sig. (2-tailed)	0.104	0.619	0	0	0.260	0.469
	N	24	16	24	16	24	16
Uric acid	Correlation coefficient	0.078	0.124	0.239	-0.195	1.000	1.000
	Sig. (2-tailed)	0.697	0.648	0.260	0.469	0	0
	N	27	16	24	16	27	16

Abbreviations: NLR – neutrophil-to-lymphocyte ratio; N – number of patients; Sig. (2-tailed) – 2-Tailed statistical significance; Spearman's rho – Spearman's rank correlation coefficient

Table V. Correlations between neutrophil-to-lymphocyte ratio and fibrinogen, total calcium, total cholesterol, triglyceride of patients

Parameter	Spearman's rho	NLR		Fibrinogen		Total calcium		Total cholesterol		Triglyceride	
		Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
NLR	Correlation coefficient	1.000	1.000	0.113	0.135	0.109	0.144	0.087	-0.383	0.057	-0.227
	Sig. (2-tailed)	0	0	0.701	0.710	0.736	0.758	0.671	0.144	0.797	0.415
	N	27	17	14	10	12	7	26	16	23	15
Fibrinogen	Correlation coefficient	0.113	0.135	1.000	1.000	0.143	0.232	-0.517	0.287	-0.282	0.239
	Sig. (2-tailed)	0.701	0.710	0	0	0.787	0.658	0.070	0.421	0.329	0.507
	N	14	10	14	10	6	6	13	10	14	10
Total calcium	Correlation coefficient	0.109	0.144	0.143	0.232	1.000	1.000	0.516	0.500	0.517	-0.252
	Sig. (2-tailed)	0.736	0.758	0.787	0.658	0	0	0.104	0.253	0.126	0.585
	N	12	7	6	6	12	7	11	7	10	7
Total cholesterol	Correlation coefficient	0.087	-0.383	-0.517	0.287	0.516	0.500	1.000	1.000	0.399	0.243
	Sig. (2-tailed)	0.671	0.144	0.070	0.421	0.104	0.253	0	0	0.066	0.383
	N	26	16	13	10	11	7	26	16	22	15
Triglyceride	Correlation coefficient	0.057	-0.227	-0.282	0.239	0.517	-0.252	0.399	0.243	1.000	1.000
	Sig. (2-tailed)	0.797	0.415	0.329	0.507	0.126	0.585	0.066	0.383	0	0
	N	23	15	14	10	10	7	22	15	23	15

Abbreviations: NLR – neutrophil-to-lymphocyte ratio; N – number of patients; Sig. (2-tailed) – 2-Tailed statistical significance; Spearman's rho – Spearman's rank correlation coefficient

When compared with the general population, patients with chronic kidney disease experience a more frequent and important cardiovascular disease, as the rate of cardiovascular events is inversely correlated to renal function, particularly for individuals in categories 4 and 5 of CKD. However, most patients do not progress to end-stage renal disease due to premature death as a consequence of cardiovascular complications [38]. Cardiovascular diseases related to renal impairment includes hypertension, coronary artery disease, heart failure, arrhythmias and lower extremity artery disease [39].

Early recognition and control of risk factors for atherosclerotic disease is mandatory in chronic kidney disease patients.

The occurrence of low level chronic inflammation during chronic kidney disease advancement is a determining factor for atherosclerosis progression.

Chronic kidney disease related inflammation bears a specific patho-mecanism that may enhance atherosclerosis through elevated generation of carbamylated low density lipoproteins, proinflammatory high density lipoproteins, cholesterol crystals, inflammasomes, and calciprotein particles [34,40].

Neutrophil-to-lymphocyte ratio as a marker for inflammation can be an important tool in this context, as it has already emerged as an important marker for atherosclerotic disease, vascular calcification and also as a prognostic factor in CKD hypertensive patients [15,18,27]. Furthermore, neutrophil-to-lymphocyte ratio is a predictive factor for vascular calcification in end-stage renal disease [27].

Computation of neutrophil-to-lymphocyte ratio is a very simple mathematical and inexpensive method using results from automated white blood cell count when compared with assessment of any other inflammatory markers. This retrospective study identified a positive correlation between NLR and aortic arch calcification in hypertensive non end-stage CKD patients. Additionally, elevated alkaline phosphatase values and increased erythrocyte sedimentation rate had a positive association with aortic arch calcification; this is an expected result as alkaline phosphatase and erythrocyte sedimentation rate increase with ageing and fall in glomerular filtration rate. Our group with aortic arch calcification was comprised of older patients compared to the group without aortic arch calcification and had a more advanced renal disease.

Additionally our study has some limitations: is a small sample size single center retrospective study; routine phosphate – calcium characterization and intact serum parathormone levels (related to calcium metabolism) were not available. Regarding neutrophil-to-lymphocyte ratio distribution in the different comorbidities groups, we refrain from forming definite conclusions as a result of the limited number of patients included in the study.

To the best of our knowledge this is the first Romanian study which explores NLR as a novel inflammatory marker in hypertensive patients with less advanced CKD.

Conclusions

Neutrophil-to-lymphocyte ratio may be viewed as a risk factor for vascular calcification even in patients with less advanced kidney disease. The small number of individuals included in our study restrains us from reaching definite conclusions. Further prospective large scale studies, including primary care databases are mandatory in order to validate this accessible inflammatory marker as risk factor for arterial calcification in patients with CKD.

Conflicts of Interest

None to declare.

Authors' contribution

Claudia Floriana Suciu, MD, PhD student (Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation; Visualization; Writing –original draft; Writing – review & editing)

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RESEARCH ARTICLE

A Prospective Study about the Influence of Selenium Based Supplements on the Autoimmune Process Evolution and the Health-Related Quality of Life in Patients with Chronic Autoimmune Thyroiditis

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Objective: The purpose of this study was to investigate the benefits of two different Selenium based supplements on patients with chronic autoimmune thyroiditis. **Methods**: We conducted a prospective study on 50 patients with chronic autoimmune thyroiditis, who were divided into three different treatment groups, one group taking Selenium 100 µg, one Procor T (a combination of Selenium 100 µg and other elements like copper, Zinc and Q10 Conenzyme) and one control group taking Placebo pills. We measured on two follow up visits the antibody levels (anti-thyroidperoxidase- TPO Ab) and offered each patient a standardised questionnaire regarding the thyroid-related quality of life (THYPROro). **Results**: At the 6 months follow up visit there was a statistically significant decrease in the antibody levels for each treatment group compared to the base levels. The THYPROro questionnaire scores showed an improvement in most aspects regarding the quality of life as well, but there was no significant difference between the placebo and the treated groups in the magnitude of this improvement. **Conclusions**: Based on our results, we could not identify a certain benefit in improving quality of life with the supplementation of Selenium, as the improvements were at a similar level for the patients who took Placebo pills. Further studies with more patients, as well as taking the Selenium defficiency in consideration (by measuring the basal serum level of Selenium for each patient) would be required to find the target group of patients who could have most benefits of Selenium-based supplementation.

Keywords: chronic autoimmune thyroiditis, selenium, quality of life, autoantibodies, hashimoto

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Introduction

Chronic autoimmune thyroidits is one of the most common diseases diagnosed in endocrinological practice, and is also the main cause of hypothyroidism in adults. Patients with this disease present a broad spectrum of symptoms and signs, ranging from mild to severe, which can be caused both by the thyroid dysfunction (ussualy hypothyroidism) but also by unknown mechanisms through the autoimmune process itself. It is not uncommon for patients with normal thyroid function to manifest different types of symptoms, which affect the quality of life in various ways [1, 2]. Selenium is an element with multiple roles in the human body homeostasis, even though most of it's mechanisms of actions are still unknown [3, 4]. Selenium is known to have a role in the production of Selenoproteins, one of these proteins having a major role in the methabolism of thyroid hormones- iodotyronine deiodinase. This enzymes have an important role in the activation of thyroid hormones, by converting the prohormone thyroxine (T4) to the more active form triiodothyronine (T3) [5].The objective of our study was to investigate the effects of two different types of selenium-based supplements on the quality of life (measured through a standardised questionnaire) and the serum anti-thyroidperoxidase antibody levels (TPO-Ab) in patients diagnosed with chronic autoimmune thyroidits having a normal thyroid function with or without Levothyroxine (LT4) therapy.

Methods

Our study started in 2016 in the Endocrinology compartment of the Mures County Hospital. Durine a timeframe of approximatly one and a half year 50 patients were enrolled in the study. Before starting the study we obtained an approval form from the ethics comitee of the University of Medicine and Pharmacy of Targu Mures (annexed). Every patient who entered the study signed an informed consent form which contains a detailed description of the study design and implications. The inclusion criteria were the following: patients diagnosed with autoimmune thyroidits (based on elevated anti-thyroid autoantibodies- TPOAbor anti-Tg Ab) and/or sonographic aspect typical of autoimmune thyroidits; patients with normal thyroid function with or without thyroid hormone substitution(thyroid stimulation hormone- TSH and thyroxine-FT4 hormone within normal reference range). For the laboratory measurements mentioned chemiluminescence assay was used (Architect I 1000 SR Immunoassay).The reference ranges for each assay were the following: TSH: 0.35-4.94 µIU/ml, fT4: 0.7-1.48 ng/ml, TPOAb: 0-5.61 IU/ml, TgAb:

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0-4.11 IU/ml. The exclusion criteria were set as following: previous diagnosis of other thyroid diseases; history of radioactive iodine treatment, anti-thyroid medication or other therapies that could influence thyroid function; patients with other associeted severe pathology which could significantly influence the health-related quality of life; patients uncapable of adherence to the study for various reasons or who did not sign the informed consent. We divived the patients into three treatment groups through a randomisation and blinding process (trail drugs were labeled as A, B and C, with the exact supplement contained being revealed only at the end of the study to the authors): one group treated with Selenium 100 μg one tablet/day, the second group treated with a supplement called Procor T (a combination of Selenium 100 μg, Q10 coenzyme, Copper and Zync) one tablet/day, and the last group received Placebo-type tablets (tablets containing no active substances). All the mentioned trial drugs were provided by the company Sunwave Pharma.We did not lose any patient during follow-up. For the evaluation methods and the gathering of informations and data, we conducted an initial visit and two follow-up visits after 3 and 6 months. In all three visits the following investigations were conducted: anamnesis, clinical exam, TPO-Ab , FT4 and TSH serum levels, thyroid ultrasound and data obtained from the filling of the standardised Thyroid-related quality of life questionnaire (THYPROro). This instrument called THYPROro is a questionnaire made for evaluating the impact of chronic thyoird diseases on health-related quality of life. It was first created in Denmark by Dr. Torquil Watt and colaborators from Ringhospitalet University Hospital of Copenhagen [6]. Following several international studies this instrument was validated and translated into Romanian language[7]. The questionnaire contains a total of 85 questions grouped into 13 scales which cover the following: physical symptoms, mental symptoms, general well being, social life and one final question regarding quality of life as a whole. The patients can answer each question by themselves with choosing one option from 0 (none) to 4 (very much) after a short introduction. A final score will be calculated for each scale which can range from 0 to 100 (the higher the score the greater the negative impact on the quality of life). For the analysis of the statistical difference between the means of different groups we used the non-parametric tests Friedman and Mann-Whitney. We set the level of statistical significance as the standard p= 0.05.

Results

The final size of each treatment groups was set as following: Placebo group 16 patients, Selenium group 17 patients and Procor T group 17 patients. All patients included had a normal thyroid function (FT4 and TSH within reference range) with 60% of the patients being under LT4 treatment and 40% with no hormonal treatment. 72% of the patients included had a ultrasound aspect with nodules, while 28% did not present any nodules. The TPOAb antibodies were positive in 88% of the patients and 22% of the patients had positive values for TgAb (Table I).

Regarding the results of the THYPROro scores for each treatment group on all three visits, there was a statistically significant decrease (meaning an improvement in the quality of life) on most scales for all three treatment groups, however no statistically significant difference between the magnitude of this improvement when comparing each treated group with the Placebo group. (p> 0.05 for all scales) (Table II).

By analysing the results regarding the TPOAb values we noticed there was a decrease of antibody levels in all three treatment groups, but the decrease proved to be statistically significant only for the group Procor T and Placebo (p < 0.05). We also applied a Mann-Whitney test to compare the difference of the TPOAb levels between the entry- and 6 months visit between the Placebo group and each treatment group, with the results showing no statistically significant difference in both cases (Placebo and ProcorT : p = 0.95, Placebo and Selenium: p = 0.09) (Figure 1).

The results of the THYPROro scores from the last question of the questionnaire about the general impact of the thyroid disease on the quality of life showed a similar pattern to the TPOAb, with a tendency to decrease, the difference being statistically significant only for the Procor T and Placebo groups (p= 0.004 and 0.0003) but not for Selenium group (p=0.46) (Figure 2).

The TSH levels did not show any statistically significant difference between the three visits on none of the treatment groups. (We applied Friedman test for assessing this aspect - p > 0,05 for every treatment group) (Figure 3).

Discussions

Some research have demonstrated that Selenium has an influence on the differentiation process of the lymphocyte T cells. Therefore increasing Selenium serum levels might produce a raise in the Ly T supressor cell population, which would lead to a decrease in the inflammatory infiltration of the thyroid and the production of autoan-

Table I. General characteristics of the patients group (DS= standard deviation)

Group size:	50
Women	49 (98%)
Men	1 (2%)
Average age	51± 11.72 SD
Patients under LT4 treatment	30 (60%)
Patients without LT4 treatment	20 (40%)
Nodular ultrasound aspect	14 (28%)
Nodule-free ultrasound aspect	36 (72%)
TPO Ab positive	44 (88%)
TgAb positive	11 (22%)
Average height	163 cm ± 6.40 SD
Average weight	72 kg ± 15.58 SD
Average BMI (Body Mass Index)	27 ± 5.67 SD
Normal weight patients	23 (46%)
Overweight patients (BMI 25-30)	12 (24%)
Grade 1 obesity patients (BMI 30-35)	10 (20%)
Grade 2 obesity patients (BMI 35-40)	5 (10%)

Table II: THYPROro average scores for each treatment group on all three visits.

PROCOR T GROUP

Scale	First screening visit	3 months- follow-up visit	6 months- follow-up visit	p*
Goiter symptoms	34.75	17.11	16.97	0.00
Hypethyroidism symptoms	35.15	22.02	17.64	0.01
Hypothyroidism symptoms	44.11	30.51	22.05	0.00
Eye symptoms	22.80	15.62	12.68	0.02
Fatigue	52.73	45.79	46.85	0.08
Cognitive problems	31.86	25.98	23.28	0.41
Anxiety	47.54	27.69	23.04	0.00
Depression	43.54	35.92	34.03	0.49
Emotional disturbances	45.75	32.18	31.37	0.001
Social life	15.80	10.66	12.13	0.4
Personal life	24.75	13.23	13.23	0.004
Sexual problems	36.02	29.41	33.80	0.479
Cosmetic problems	20.34	17.40	8.08	0.114

PLACEBO GROUP

Scale	Initial screening visit	3 months- follow-up visit	6 months- follow-up visit	p*
Goiter symptoms	47.98	23.43	20.45	0.001
Hypethyroidism symptoms	38.67	19.72	19.92	0.002
Hypothyroidism symptoms	47.65	31.25	22.65	0.001
Eye symptoms	35.35	17.58	17.38	0.002
Fatigue	54.24	43.08	45.98	0.001
Cognitive problems	43.80	26.30	24.74	0.004
Anxiety	48.17	27.86	28.65	0.004
Depression	52.67	36.38	32.58	0.007
Emotional disturbances	47.04	30.20	34.02	0.004
Social life	22.65	9.35	14.45	0.19
Personal life	41.40	16.66	14.06	0.005
Sexual problems	39.06	36.71	26.56	0.156
Cosmetic problems	23.43	9.89	9.63	0.005

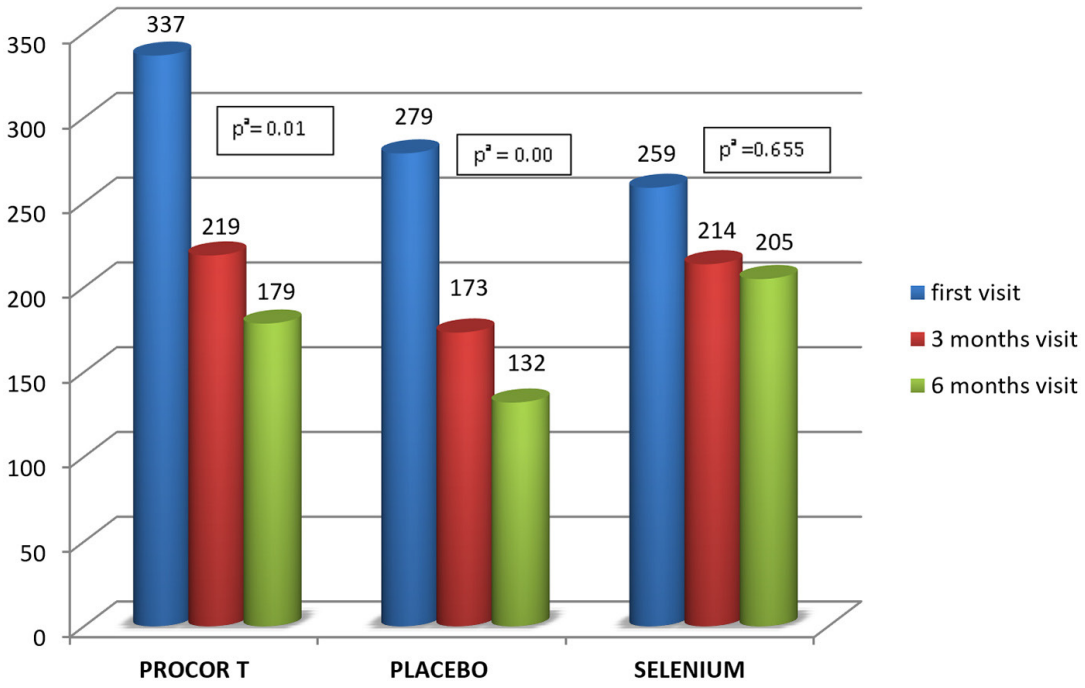
SELENIUM GROUP

Scale	Initial screening visit	3 months-follow-up visit	6 months- follow-up visit	p*
Goiter symptoms	32.48	18.44	16.04	0.00
Hypethyroidism symptoms	23.71	19.11	16.36	0.03
Hypothyroidism symptoms	29.41	16.54	15.44	0.002
Eye symptoms	13.60	12.86	8.27	0.04
Fatigue	46.63	40.75	42.43	0.136
Cognitive problems	33.32	20.09	25.24	0.024
Anxiety	42.40	27.69	31.12	0.013
Depression	44.74	34.66	35.92	0.020
Emotional disturbances	41.17	32.18	34.15	0.014
Social life	12.86	4.77	4.77	0.067
Personal life	19.36	9.80	6.61	0.013
Sexual problems	30.88	27.94	25.73	0.52
Cosmetic problems	8.08	3.67	2.45	0.072

*Friedman test was applied to calculate the statistical significance of the difference of the scores between each visit

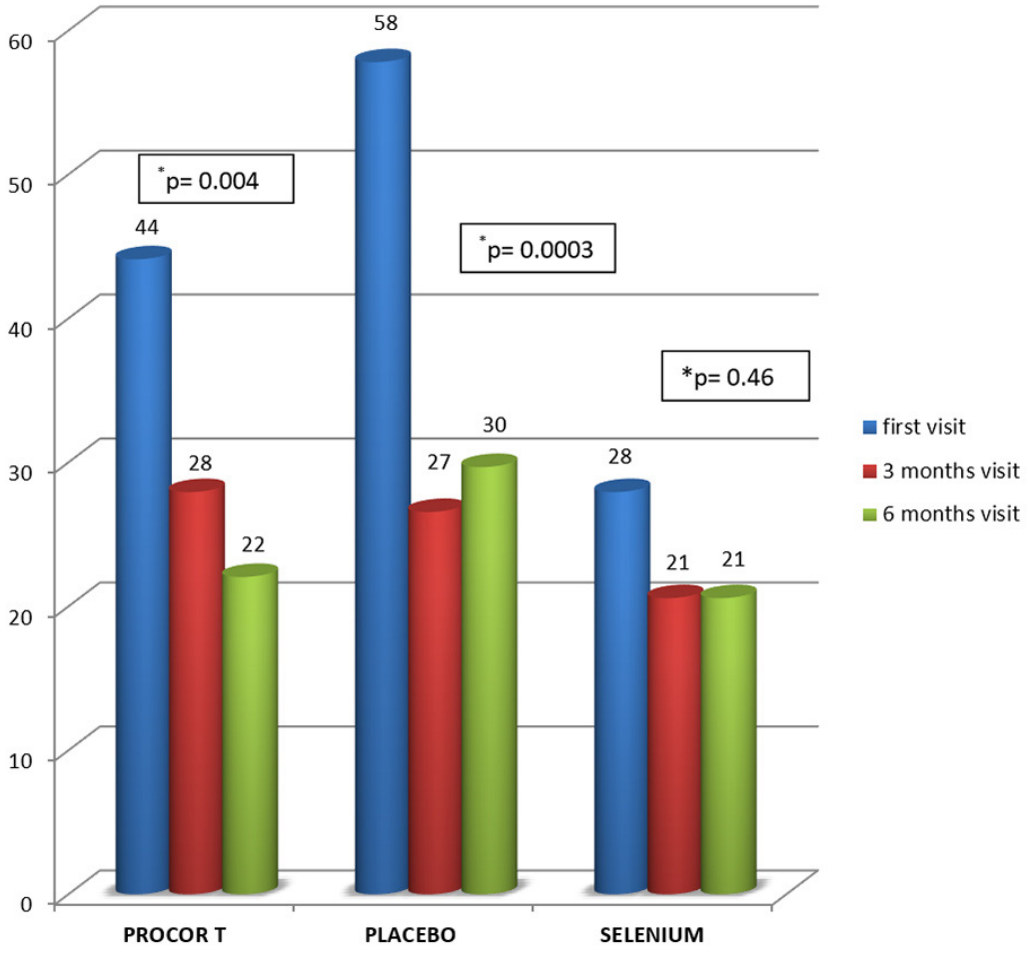
tibodies [3]. Some studies which investigated the correlation between Selenium supplementation and anti-thryoid antibody activity did not produce any conclusive results. [8, 9]. Our study showed a decrease in antbody activity, but this decrease was found not only in the treated groups, but also in the control (Placebo) group, therefore we could not find a clear causality between Selenium supplementation and antibody serum levels. Another meta-analysis published in literature showed there was a moderate decrease in antibody levels and a small improvement in patients quality of life after three months Selenium supplementation in chronic autoimmune thyroidits patients [10]. A study which also investigated the effect of Selenium supplementation in Hashimoto thyroidits concluded that the decreasing of antibody levels was significantly higher in patients who had the initial levels of TPOAb levels very high (before starting the Selenium treatment) compared to those with low-moderate serum levels. Therefore patients with very high TPOAb serum levels could have more benefits from Selenium supplementation therapy than patients with lower antibody levels [11, 12]. Also another factor which could be important for a bigger effect of Selenium therapy would be selecting patients who have a Selenium deficiency, by measuring Selenium serum levels [13].

The health-related quality of life, in our case specific to thyroid-diseases, is a subject in permanent debate in the current medical-field research. There is a tendency to ignore this subject in day to day medical practice because of the focus on somatic and objective features of diseases, but the outcome of the therapeutic act would be greatly



a Friedman test was applied to analyse the statistical significance of the TPOAb values differences between the three visits

Fig. 1. Mean values of TPOAb levels (IU/ml) for all three treatment groups on each visit.



a Friedman test was applied to analyse the statistical significance of the score differences between the three visits

Fig. 2. THYPROro mean scores on the last question regarding the general impact on quality of life.

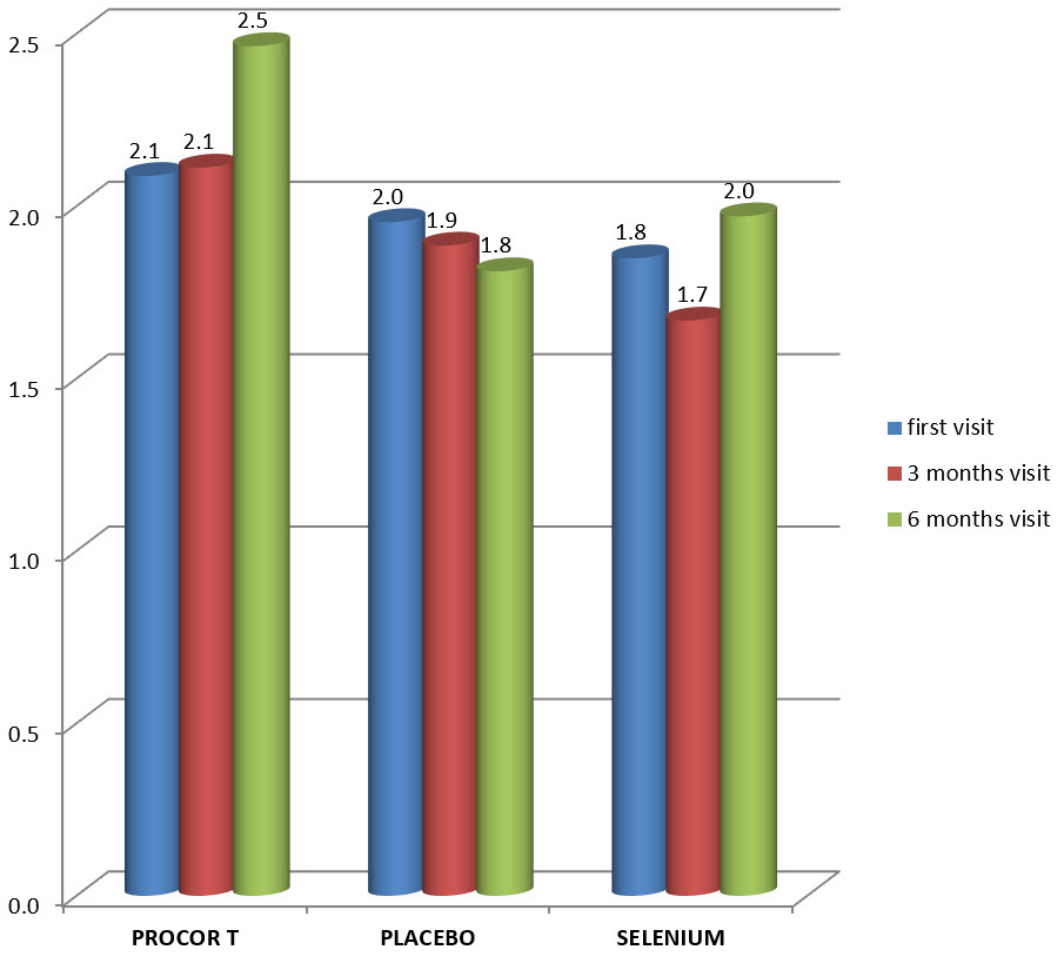


Fig. 3. Mean values of TSH (μIU/ml) for each treatment group on each visit.

improved by trying to include the quality of life aspects in the therapeutical management of patients, especially in those with chronic autoimmune diseases, like Hashimoto thyroiditis. A study from Italy evaluated the quality of life impairments in patients with various chronic thyroid diseases. An interesting finding of this study is that the quality of life in patients with autoimmune thyroiditis with normal thyroid function was significantly lower than in patients with other thyroid diseases, who presented subclinical hypo- or hyperthyroidism [14]. In our study we used the standard and validated unit called THYPROro (described in the „Methods" section of the article) as a tool to measure the health-related quality of life. The results showed a statistically significant improvement in most scales of the questionnaire for the Placebo group, with the exception of social and sexual life. In the Selenium group the results were similar to the Placebo group, with the scales showing no improvement being: cosmetic problems. social and sexual life. For the Procor T group patients showed improvements in the THYPROro scores as well, the exceptions being: depression, cognitive problems, cosmetic problems, social and sexual life. By analysing these results we could not find any relevat conclusion from our study regarding a benefit on the quality-of-life of these two Selenium-based supplements in patients with autoimmune thyroidits, as there was no statistically significant difference between the placebo and treated groups in any of the scales. We noticed that aspects related to sexual and social life had no significant changes between visits, therefore these might not be influenced by the autoimmune thyroid disease evolution too much. On the last question of the questionnaire regarding the impact of the quality of life in general, we noticed a statistically significant improvement in the score for the Procor T and Placebo groups only, and none for the Selenium group. Our findings show that there was a corellation between high TPOAb serum levels and a decreased quality of life. Other studies in literature have demonstrated there was a statistically significant corellation between high anti-thryoid antibody levels and an impaired quality of life, but no corellation between antibody levels and thyroid dysfunction [15]. These findings might indicate that the mechanisms through which the autoimmune disease affects the quality of life is not only related to the dysfunction of the thyroid. Many other insufficiently known physiopathological consquences of the autoimmune process in the human body might be responsible for the various symptoms that cause patients to feel a decline in the quality of life.

Our study was a preliminary one with multiple limitations, but potential for improvement and continuity. First

of all, the number of patients included was fairly low (50), out of whom only 44 had positive TPOAb levels (the others having positive anti-thyroglobuline levels). Another limitation is due to the fact that the initial TPOAb values range was high, as both patients with very high antibody levels (above 500 UI/ml) and patients with just mild positive levels were included. As the number of patients included was low, other limitations of our study could be noticed in several other aspects: BMI (Body Mass Index)-54% of the patients included presented a BMI over 25, so even though overweight and obesity is common in patients with autoimmune thyroidits, data regarding quality of life might have been influenced by this high percentage of obese or overweight patients. As it is known that associated diseases and medication could have a high impact on a patient's quality of life, we respected the inclusion criteria by not including patients with severe enough commorbities that would influence the quality of life results. For a more accurate investigation in future studies, the serum levels of Selenium should also be investigated before including the patients in the study, because Selenium defficiency could be a major factor that contributes to a higher benefit of Selenium-supplementation therapy in autoimmune thyroiditis patients. For a better compliance from the patients and a more facile gathering of data from the questionnaire, we recommend implementing an electronic version of the questionnaire, as data from recent studies indicated this could improve the process of researching the health-related quality of life aspects [16].

Conclusions

By analysing the results of this preliminary study we could conclude the following:

The two Selenium-based supplements which we studied did not prove to have any benefits regarding an improvement in the quality of life aspects.

Our study did not show a certain influence of the Selenium-based supplements on the TPOAb serum levels.

Most patients with autoimmune thyroidits included had a significant improvement in most aspects of quality of life between the entry - and 6 months follow-up visit, therefore the placebo-effect on some of these symptoms could have been beneficial.

The high antibody serum levels seem to be correlated to impaired quality of life, although the mechanism of this relation is not yet known, as none of the patients had any thyroid dysfunction during the study.

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Authors' contribution

Maximilian Cosma Gliga (Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing – original draft; Writing –review & editing)

Ionela Maria Pascanu (Conceptualization; Formal analysis; Investigation; Project administration; Supervision; Validation; Visualization)

Camelia Gliga (Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Supervision; Validation; Visualization; Writing – review & editing)

Ancuta Elena Zahan (Conceptualization; Formal analysis; Investigation; Methodology; Software; Supervision; Validation; Visualization)

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RESEARCH ARTICLE

Urinary Sodium/Potassium Ratio in Acute Kidney Injury Accurately Differentiates Prerenal Azotemia from Acute Tubular Necrosis

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Objective: To develop a more accurate, cost effective, non-invasive test to differentiate between pre-renal renal failure (PRA) and acute tubular necrosis (ATN) in acute kidney injury (AKI). **Methods**: Urine sodium/potassium (Na/K) ratios were compared with fractional excretion of sodium (FeNa) and renal failure index (RFI) as well as other commonly used indices to differentiate patients with PRA from ATN. Patients with a rise in serum creatinine > 0.5 mg/d identified from medical records for a six- to eighteen-month period, were reviewed and categorized either as PRA or ATN based on presenting findings, course in hospital or renal biopsy. All patients had urinary sodium and potassium, creatinine, and serum creatinine done. **Results**: The Na/K was < 1 in PRA and > 1 in ATN, correctly identifying all 42 cases of PRA and all 28 patients with ATN. The FeNa was >1 and misdiagnosed 9 of 42 patients with PRA and was >1 and correctly diagnosed all patients with ATN. The RFI was >1 and misdiagnosed 11 of 42 patients with PRA but was >1 and correctly diagnosed all patients with ATN. The BUN/creatinine ratio, urine sodium concentration and U/P creatinine ratio all had a very poor correlation with the correct diagnosis. **Conclusion**: The Na/K ratio correctly diagnosed all 42 cases of PRA and all 28 cases of ATN. It is easy to do, is cost effective, non-invasive, and is useful for following patients with PRA to see if and when they develop ATN.

Keywords: acute renal injury, prerenal azotemia, tubular necrosis

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Introduction

The differential diagnosis of acute kidney injury (AKI) has been of major interest for nephrologists and for all physicians caring for patients with acute medical problems for over 50 years. This is not just an academic exercise but has significant medical consequences specifically with regard to fluid balance and drug administration. The major problem in AKI is differentiating between a poor perfusion state identified as pre-renal azotemia (PRA) and acute tubular necrosis (ATN) which are the major etiologies of AKI. Acute glomerulonephritis in the adult is rare and has a rather classical urinary sediment and a much different clinical presentation. Urinary obstruction in adults can be reliably diagnosed with renal ultrasound done 24-48 hours post onset of obstruction. Most patients are anuric but some high grade obstructions may occasionally be polyuric. Acute interstitial nephritis (AIN) is much less common than ATN and must be distinguished from ATN using parameters other than renal electrolyte indices.

In an attempt to differentiate between PRA and ATN, in 1970 Bricker [1] proposed measuring the urine sodium. If the urine sodium was <20 mEq/l this was indicative of PRA and >40 mEq/l suggested ATN. Over the next 10 years multiple researchers proposed numerous methods of differentiating between PRA and ATN. These included changing the urine sodium concentrations to < 20 mEq/l for PRA and > 30 mEq/l for ATN [2], the renal failure index (RFI) defined as U_{Na} x P_{Cr}/U_{Cr} [3], calculating fractional excretion of Na (FeNa) [4], and finally the calculation of the fractional excretion urea (FEUN) [5].

All of the above methods have some overlap and lack specificity. Furthermore the more accurate RFI and FE_{Na} require blood samples and FE_{Na} requires the measurement of the serum and urine creatinine and a timed urine collection. The urine creatinine is notoriously inaccurate, due to problems with collections of urine even in catheterized patients, and increased tubular secretion of creatinine in renal failure [6].

In this paper we are proposing a new method of differentiating between PRA and ATN. This method does not require blood samples, rather, only the collection of the spot urine for determination of Na and K concentration. Because of its simplicity and low cost it can be used repeatedly to monitor PRA to determine if and when the patient may be developing ATN. Its sensitivity and specificity are 100%.

Methods

This study was approved by the Institutional Research Review Board (IRRB) and patient consents were waived since it was a retrospective study with only a chart review and no patient identity was revealed. Over 200 patient charts were reviewed over a six-month period at Loma Linda University Medical Center (LLUMC), and over an 18 month period at Redlands Community Hospital (RCH). The study was based on patient data from LLUMC and RCH laboratories. Patients with a rise in creatinine >0.5 mg/dl were evaluated for study. Exclusion criteria: patients who were catabolic, on steroids, malnourished, had a persistent

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high fever, or had blood in the gastrointestinal (GI) tract causing a rise in the BUN and not in the serum creatinine, patients with obstructive uropathy, patients taking distal sodium blockers affecting K^+ secretion, patients on massive loads of HCO_3^-, patients with chronic renal failure, patients with a rise in BUN, but a rise in serum creatinine less than 0.5 mg/dl were excluded from study. Patients who did not have serum and urinary electrolytes done on the same day or had no urinary electrolytes done and had to be eliminated due to a lack of data, or patients with lack of data to support a diagnosis of either PRA or ATN were also excluded. Only patients with an acute rise in serum creatinine > 0.5 mg/dl were included in the study.

A total of 42 patients were determined to have PRA based on their clinical diagnosis and course in hospital. Their diagnoses were typical of most poor perfusion states; cirrhosis, hepatorenal syndrome, congestive heart failure, dehydration, or postoperative volume depleted states. The mean urine volume of these patients at the time of measurement of the urine Na/K ratio, the FE_{Na}, and RFI was 860 ml/day. The urine outputs varied from 22 ml/day to 3,475 ml/day. The higher urine volumes were seen in the early recovery phase of PRA when some of the patients were on loop diuretics. Monitoring the daily urine output in many of these patients with PRA was of little value, since many had severe liver disease and urine outputs stayed low as did those patients with severe congestive heart failure who did not survive. Those patients who survived and did well had urine outputs that varied from day to day. In this patient population, 53% were female and 47% male. In 22 of these patients, ages ranged from 36-84 years with a mean age of 63 years. Comorbidities included diabetes mellitus in 27%, alcoholism, 12%, hypothyroidism, 13%, COPD, 13%, and cancer, 13%.

The urinalyses on these patients were benign with occasional one plus protein and 2-5 rbc (red blood cells) and wbc (white blood cells) per hpf (high power field). Urinary electrolytes, sodium, potassium and chloride and urinary urea and creatinine were measured on a spot urine sample close to the time when the serum values were done.

The time to recovery in those patients that survived or did not develop ATN varied from 4 days to 18 days with a mean recovery time of greater than 8.5 days. The mean drop in serum creatinine was 1.34 mgs/dl. Fourteen of the 38 patients were either on furosemide or bumetanide with a mean urinary Na/K ratio of 0.29, which was slightly less than the 0.32 Na/K ratio in those patients who were not on diuretics (see Table I).

Twenty-eight of the patients were determined to have ATN based on their clinical presentation and course in hospital. Four had prolonged shock, seven had sepsis, one of whom received gentamycin. Four received IV contrast in the presence of, or prior to, liver failure, surgery for abdominal aortic aneurysm (AAA), and one had stage IV immunoblastic sarcoma. Two received nephrotoxic antibiotics and two had acute rhabdomyolysis, the remainder were due to SLE with anaphylactoid vasculitis secondary to penicillin (biopsy proven ATN), acute lymphocytic leukemia with ATN at post, multiple myeloma, post severe seizure disorder and post cerebral hemorrhage.

Urine volumes varied considerably depending on the severity of the kidney injury (polyuric or oliguric ATN). Some patients presented with ATN and others developed ATN in the hospital. Urine volumes at the time of study varied from a low of 10 ml/day to a high of 4,540 ml/day. The higher urine volumes were seen in patients with mild AKI with ATN (polyuric) or in the early recovery or diuretic phase of ATN. Of the 18 patients where volumes were recorded, 7 were oliguric (<500 ml/d) and 11 were polyuric. The mean time to recovery in those patients that survived was > 7 days with a range of 4 days to discharge on hemodialysis. None of these patients were on diuretics. The mean drop in the serum creatinine was 4.42 mgs/dl.

In 27 of the 28 patients, there were 14 female and 13 male patients with a mean age of 47.9 years in 20 of the 28 patients. Their ages ranged from 1 year to 81 years. Comorbidities included alcoholism 15%, cancer 15%, obesity 1 case.

Serum creatinines were measured daily to every other day during the first 10 days of admission, and frequently after that in all patients. Measurements of urinary indices was done on admission for patients with an elevated serum creatinine or on patients whose serum creatinine increased greater than 0.5 mgs/dl during hospitalization.

Urinalyses were benign with a trace protein and 0-3 rbc or wbc/npf.

Fluid resuscitation with IV NaCl was vigorous for those patients in septic shock often with vasopressors or with large urine outputs, and restricted for patients who were fluid overloaded, in CHF or severely oliguric.

A third group of 7 patients initially presented clinically with poor perfusion states who subsequently developed ATN. Serial measurements of serum and urinary electrolytes were obtained in these patients documenting the evolution from a PRA to ATN. Two of these patients had thoracic aortic aneurysms that had ruptured, and one was an abdominal aortic aneurysm (AAA), all survived post surgery. Three of the other patients had severe acute complicated cardiovascular problems with septic shock, GI bleeding, and liver failure.

Statistical analysis was done using both the one-sample Wilcoxon signed rank test and the student t test. The P value for the urine Na/K ratio, the F_{Na} and the RFI were calculated. See Tables I and II.

Results

PRA

Table I summarizes the serum and urine laboratory data from the 42 patients who had PRA. The serum sodium, chloride and potassium were typical of most renal failure patients and are not included in the table. The mean se-

Table I. Poor Perfusion. In Table I are shown the urine Na, K, and Cl all in mEq/l and the urine creatinine in mgs/dl. Four ratios are shown, the urine Na/K ratio, the urine Cl/K ratio, the urine/plasma ratio of creatinine and the serum BUN/creatinine ratio. Also shown are the fractional excretion of Na (FENa), and the renal failure index (RFI) (sodium in urine ÷ urine Cr/plasma Cr). All 42 patients had a poor perfusion state.

Case #	Serum BUN mgs/dl	Serum Cr mgs/dl	Urine Na/K	Urine Cl/K	FNa	RFI	U/P Creat	BUN Creat	Loop Diuretic
1	132	3.6	0.26	<0.32	0.18	0.26	46	37	
2	106	2.9	<0.12	<0.18	0.18	0.23	36.6	36.5	Yes
3	30	1.6	0.89	0.93	0.69	1.1	65.2	187.5	Yes
4	45	2.9	<0.19	<0.28	0.08	1.1	90.3	15.5	
fv5	50	2.2	<0.2	<0.24	0.32	0.45	24.6	22.8	Yes
6	53	2.1	0.28	<0.42	0.08	1.0	9.4	25.2	
7	93	6.5	0.16	<0.24	0.35	6.5	23	14.3	
8	93	1.6	0.16	<0.25	0.14	0.18	47.5	58.1	
9	42	3.0	0.74	1.0	1.09	2.2	19	14	
10	48	2.9	0.59	0.57	1.83	2.2	14.3	16.6	
11	180	6.1	0.45		1.39	17	10.9	29.5	
12	38	1.9	0.1	<0.14	0.5	0.59	186.9	20	Yes
13	159	3.4	0.45	0.38	1.39	1.9	10.9	46.8	Yes
14	101	3.6	<0.14	<0.21	0.32	0.43	23.1	28.1	Yes
15	40	2.8	0.32	<0.44	0.5	0.27	16	38	
16	63	2.2	<0.13	<0.19	0.2	0.26	35.4	28.6	Yes
17	76	6.7	0.53	0.74	1.3	1.7	13	11.3	
18	107	4.5	0.18	<0.25	0.35	0.47	23.6	23.8	Yes
19	63	6.5	0.3		0.66	0.88	20.5	9.69	
20	77	2.9	0.49	0.56	1.88	2.5	11.7	26.6	
21	25	1.8	0.21	<0.29	0.18	0.16	68.9	13.9	
22		5.2	0.25	0.63	1.01	1.3	13.8		
23	38	1.0	0.17	0.25	0.17	0.1	40	38.0	Yes
24	109	2.8	0.18	0.27	0.35	0.46	21.8	38.9	
25	66	1.6	0.27	0.41	0.24	0.34	29.4	41.25	Yes
26	49	3.0	0.61	.91	1.05*	2.13*	7.7*	16.33*	
27	76	4.6	0.19	0.6	1.02*	1.23*	8.11*	16.5*	
28	146	4.5	0.34	0.71	0.26	0.38	26*	32.4	
29	47	4.1	0.3		0.4	0.47	21.49*	11.46*	
30	109	2.7	0.2	0.3	0.48	0.67	14.96*	40.37	
31	34	2.6	0.15	0.21	0.18	0.26	50	13*	
32	45	3.2	0.46	0.75	0.58	0.8	43.6	14*	
33	34	2.6	0.17	0.21	0.19	0.26	50	13.1*	
34	65	2.7	<0.53	0.95	0.1	0.14	72.2	24.1	Yes
35	53	2.2	<0.27	0.42	0.66	0.09	109.1	24.1	
36	125	14.8	0.85	1.14*	0.89	1.18*	17.86*	8.45*	
37	79	2.5	0.27	0.4	0.19	0.27	37.36*	31.6	
38	66	5.2	0.35	0.43	0.13	0.16	79.81	12.7*	
39	30	2.4	0.19	0.32	0.18	0.25	39.54	12.5*	
40	126	4.5	0.3	0.33	0.24	0.32	31.11*	28	
41	51	4.9	0.42		0.54	0.73	28.78*	10.4*	
42	147	2.4	0.3	0.23	0.43	0.6	36.9*	61.25	
Mean	74.8	3.8	0.320	0.45	0.543	1.300	37.5	19.7	
SD			0.199	0.263	.543	2.728	32.882		
P value			<.0001	<.0001	<.0001	<.058			

rum K was at the upper range of normal and varied from a low of 3.4 to a high of 6.4 mEq/l. The BUN varied from a low of 25 to a high of 180 mg/dl. The BUN determination was not done in 1 patient. The serum creatinine varied from a low of 1.6 mg/dl to a high of 14.8 mg/dl.

The urine chemistries were of considerable interest. The urine sodium varied from a low of 4 to a high of 63 mEq/l. Eleven of the 42 patients had a urine sodium of > 20 mEq/l. The urine chloride paralleled the urinary sodium, however the urinary potassium was always greater than either the urinary sodium or chloride concentration and varied from a low of 16.4 mEq/l to a high of 105 mEq/l (see Table I).

The major reason for this study was to compare the urinary sodium/potassium ratio (U_{Na}/U_K) ratios with all other indices commonly used in the evaluation of AKI. In all 42 cases of PRA the U_{Na}/U_K was less than one, with ratios varying from 0.1 to 0.89 and a P value < .0001. The urinary chloride/potassium ratio (U_{Cl}/U_K) was also very low with a mean of .45. However it was not as consistent as the U_{Na}/U_K ratio and values varied from 0.18 to 1.14. The urine to plasma ratio of creatinine (U/P creat) ranged from a low of 7.7 to a high of 186.8. In 13 of the patients the U/P creatinine was less than 20 and in 28 of the patients the ratio was < 40. The mean BUN/Cr ratio was 25, and 17

Table II. ATN (Acute Parenchymal Renal Disease). Table II shows the same indices described in Table I but for the 28 patients with acute tubular necrosis.

Case #	Serum BUN mgs/dl	Serum Cr mgs/dl	Urine Na/K	Urine Cl/K	FNa	RFI	U/P Creat	BUN Creat
1	60	6.1	2.0	2.14	3.2	3.14	17.54	10.2
2	57	5.8	1.41	1.65	6.8	9.03	7.2	10
3	48	4.3	1.69			1.06	13.02	11.16
4	57	1.5	3.73	4.57		38.6	2.93	38
5	53	4.7	7.08	7.31	15	20.4	4.47	12.83
6	125	7.9	3.12	<0.9	4.9	6.6	7.59	15.82
7	30	2.0	6.64	3.96	11.73	17.5	9.5	15
8	63	2.6	2.07	2.87	16.7	23.8	3.85	24.23
9	85	5.5	5.4	4.2		18.6	3.4	15.44
10	84	5.5	3.67	4.62		32.1	2.88	15.3
11		1.8	1.12	1.61		1.7	32.2	
12	86	8.2	2.1	1.3		19	3.9	10.5
13		4.8	5.14	5.48		20.8	5.21	
14	46	3.4	1.68	1.19		3.1	20	13.5
15	65	2.3	1.28	1.53		6.9	8.7	28.3
16	125	8.7	4.73	<.99		12.7	5.63	14.1
17		4.2	2.75	4.08		6.3	10.48	
18	42	32	2.83	2.44		11.2	9.06	13.1
19	82	7.8	4.31	3.94	12.1	16.4	4.23	10.5
20	35	5.4	2.27	1.58	30.8	4.26		6.48
21	125	16.8	1.14	1.14	3.7	4.66		7.44
22	23	2.1	3.8	2.04	1.09*	1.51		10.95
23	120	3.8	2.77		3.6	5.13		31.58*
24	75	2.6	1.28	0.78*	2.54	3.54		28.85*
25	23	2.7	5.45	6.23	22.85	31.6		8.5
26	25	2.2	9.39	9.51	3.85	5.2		11.36
27	54	3.0	3.44		6.9	9.62		18*
28	74	8.7	5.2	2.13	5.6	6.92		8.51
Mean	61.6	5.3	3.86	3.34	8.99	8.05		14.63
S.D.	40.1	4.8	2.57	3.14	10.43	9.11	7.19	9.47
P value			<.0001	<.0001	<.0001	<.0001		

of the patients had a ratio < 20. Nine of the patients had a FE_{Na} > 1.0 with values ranging from 0.08 to 1.83 with a P value also < .0001. Urine volumes varied from 22 ml/day to 2100 ml/day. Most of those with larger volumes were on a loop diuretic. The loop diuretics did not appear to have any effect on the U_{Na}/U_K. The renal failure index (RFI) in these 42 patients had a low of 0.1 to a high of 17.14 and 13 of the 42 had a RFI > 1, P value 0.058 (See Table I).

ATN

In Table II are shown the same parameters, that are depicted in Table I, for the 28 patients with ATN, all of which had clinical diagnoses and courses consistent with ATN. The serum sodium and chloride were similar to the normal patients. Serum K varied from a low of 2.8 to a high of 5.8 mEq/l. The BUN varied from 23 to 125 mg/dl. Three patients did not have a BUN drawn on days when urine electrolytes were done. The serum creatinine varied from 1.5 to 16.8 mg/dl. Many of these patients were 65 to 80 years of age with presumably poor muscle mass which may have accounted for the less than expected rise in creatinine, but was still higher than the mean 3.62 mg/dl in the PRA patients.

The urine sodium concentration varied from a low of 39 to a high of 166 mEq/l. All but one of the patients had a urinary sodium concentration > 40 mEq/l. The mean urine chloride concentration was 64.2 mEq/l and generally paralleled the urine sodium concentration. Three patients did not have a urine chloride measurement and 6 patients had urine chlorides much less than the urine sodium. The mean urinary creatinine was 41.7 mg/dl and varied from a low of 4.4 mg/dl to a high of 145.6 mg/dl.

As expected the urinary indices were markedly different from the patients with PRA. The mean U_{Na}/U_K was 3.48 as compared with a mean of 0.33 for PRA. The ratios ranged from a low of 1.12 to a high of 9.39 and no patients had a ratio < 1.0, P value < .0001. Similarly the U_{Cl}/U_K ratios had a mean of 3.05 but in 3 of these patients the ratios were slightly less than 1.0. The U/P creatinine ratio ranged from a low of 1.33 to a high of 17.54. The mean BUN/creatinine ratio was 15.55 with 8 patients having a BUN/Cr of > 15.0. The FE_{Na} in the 16 patients with data available for calculation varied from 1.09 to 30.8. The RFI varied from a low of 1.06 to a high of 38.6. No values were < 1 for either the FE_{Na} or the RFI and both had P values < .0001. The urine output ranged from 100 ml/d to a high of 2420 ml/day. Once again, loop diuretics appeared to have little effect on the U_{Na}/U_K.

PRA Developing into ATN

Seven other patients had clinical courses consistent with PRA that evolved into ATN. Serial measurements of se-

rum and urinary electrolytes and indices depict their progression (Figure 1). Collections on day 1 and 2 were taken when the patient had PRA while collection 3 was done on day 3 when the patients had developed ATN.

The U_{Na}/U_K was .36 on day 1 (PRA) and increased to 2.9 on day 3 (ATN). The FE_{Na} had a mean of 0.86 on day 1 during PRA and increased to 4.18 when ATN developed. The renal failure index in the patients with poor perfusion had a mean value of 1.67 on day 1 during PRA. When these same patients developed ATN on day 3 their RFI had a mean of 13.7.

Since we did not measure the urine urea, we were unable to calculate and therefore unable to compare the fractional excretion of urea with the U_{Na}/U_K and other renal indices. However, the fractional excretion of urea requires the simultaneous measurement of both serum and urine urea and creatine concentrations.

Discussion

In hospital AKI is a common complication of many different disease states including sepsis, shock, toxic exogenous and endogenous substances, dehydration, CHF, acute and chronic liver failure, as well as many other causes. Recent studies of long-term outcomes of AKI secondary to ATN [7] have shown increasing chronic renal failure (CRF) as a long-term complication, particularly in the elderly, and an increased mortality during the acute hospitalization [8,9,10,11]. In the acute hospital setting it is also important to differentiate between ATN and PRA since the treatment with fluids and various medications will be altered by the underlying cause of the AKI. For these reasons it is important to differentiate between PRA and ATN. Obviously, a renal biopsy would differentiate between these conditions and was one of the modalities utilized in the 50's and 60's to make this diagnosis but is now reserved for patients in whom the cause of AKI is obscure [12].

In 1970, Bricker [1] proposed, based on the difference in the pathophysiology of PRA and ATN, that the urine sodium should be less than 20 mEq/l and often less than 10 mEq/l in PRA and greater than 40 mEq/l in patients with ATN[1]. Other authors proposed that a urine sodium of < 20 mEq/l for PRA and > 30 mEq/l for ATN should be used [2]. However, it was noted that there was considerable overlap between PRA and ATN, so other investigators looked for tests that would more accurately discriminate between these two conditions.

In 1967 Handa and Morrin [3] proposed measuring the RFI which was largely dependent on the fractional excretion (FE_{Na}) of sodium proposed by Espinel [4] and generally paralleled the FE_{Na}. These indices proved to be much more accurate than the urinary sodium concentration, BUN/creatinine ratio or measurements of the urine osmolality. Miller and Anderson using the RFI and FE_{Na} found very little overlap between PRA and ATN [13]. However they did find some overlap in patients with non-oliguric acute renal injury. Anderson et al. found intermediate values between PRA and oliguric ATN in non-oliguric AKI patients [14].

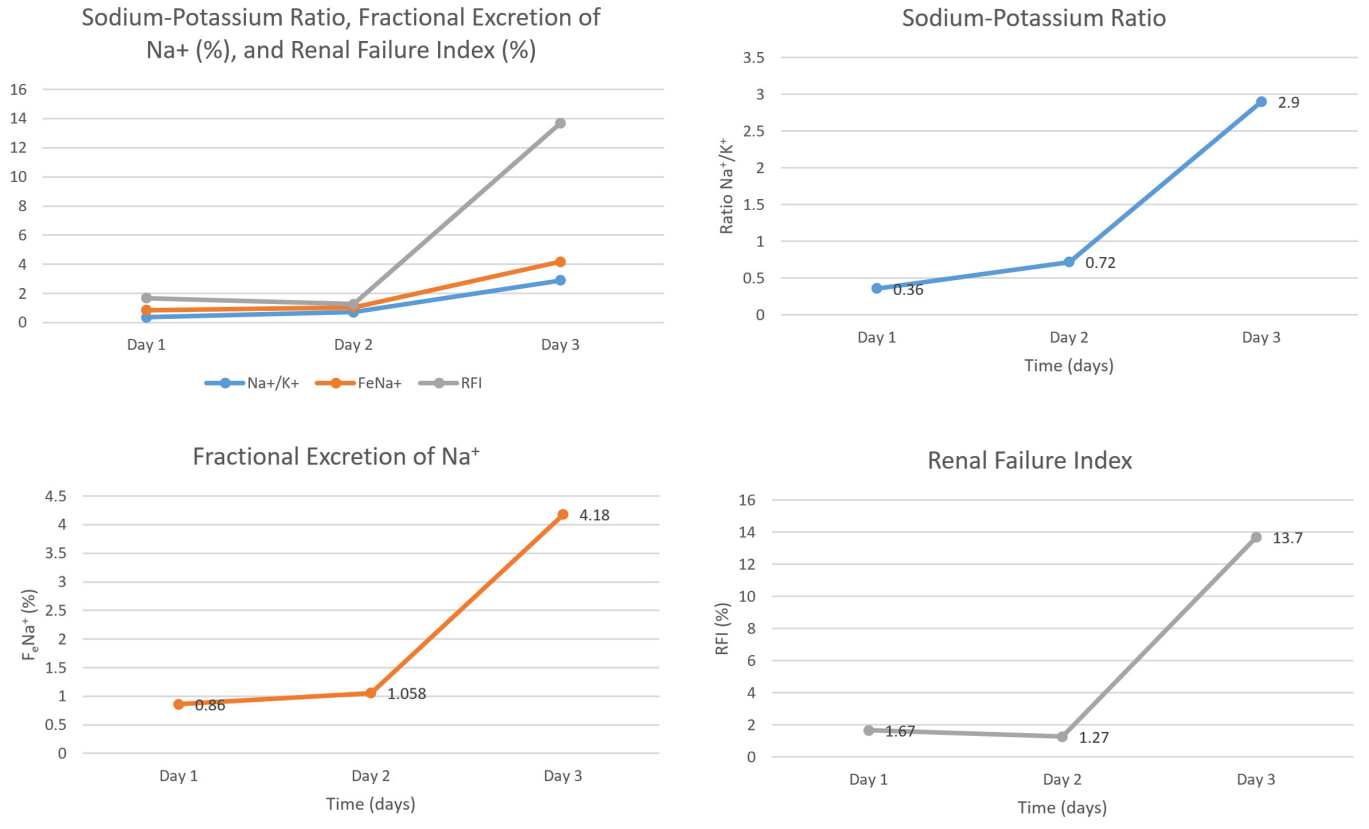

Fig.1. The results of the same 3 indices (U_{Na}/U_K, F_{Na}, RFI) used in the 7 patients who presented with severe PRA which developed into ATN. Days 1 and 2 were prior to the development of ATN while day 3 was during AT

Carvounis et. al. [5] proposed measuring the fractional excretion of urea (FEUN) in an attempt to differentiate a prerenal state from ATN. If the FEUN was < 35% the patients had PRA. In the 102 patients they studied 50 had PRA and some of these patients were on diuretics, 25 had ATN. The FEUN was more sensitive than the FE_{Na} in differentiating prerenal azotemia from ATN especially if the PRA patients had received diuretics. In their study they also looked briefly at the U_{Na}/U_K ratio. It was less reliable, but only summary data was presented and was contrary to our findings.

In a well-designed and carefully controlled study on female Marino ewes, studied before and after the onset of sepsis, Langenburg et. al. [15] concluded that "urine chemistries and indices are unreliable in sepsis and probably in other pathophysiological states." However, our data on the U_{Na}/U_K index was a reliable indicator in differentiating PRA from ATN in all causes of PRA and ATN including sepsis.

In the most recent edition of Brenner and Rector [16] a table listing all of the measurements used to differentiate PRA from ATN is presented. Nine different tests or calculations were used to try to differentiate between PRA and ATN. Other researchers [18] have noted that changes in the serum creatinine and on urine output do not identify early changes of intrinsic kidney injury which may be the most opportune time for pharmacological intervention. Coca et al., Moriates et al., Pickering et al., and Cruz et al. [19,20,21,22] have measured multiple urinary biomarkers to determine if and when tubular injury occurs. The four biomarkers studied were: kidney injury molecule 1, interleukin 18, cystatin C and neutrophil gelatinase-associated lipocalin (NGAL). Their studies were focused on trying to predict AKI, primarily ATN, by a rise in the biomarker before a rise in serum creatinine. The rise in the biomarker was attributed to severe ischemia, necrosis or inflammation of the renal tubular cells causing cell damage. The studies were prospective and did not look at patients presenting with renal failure or patients with pure poor perfusion such as dehydration, heparenal syndrome, etc. The biomarkers were also elevated in renal inflammatory states and chronic kidney disease. These biomarkers are expensive and have not been validated on a large population of AKI patients. While many of these biomarkers are increased by tubular injury they are also increased by other disease states, many of which accompany or cause ATN (23). Further studies need to be done on these biomarkers on a patient population with both ATN and PRA to determine if they can accurately differentiate between these two disease states of AKI.

A simple inexpensive test that would accurately differentiate between PRA and ATN, cause less patient discomfort, aid in the treatment, and at the same time be cost effective, should be of major interest.

A careful understanding of the pathophysiology of PRA and ATN should be of help in devising such a test. In PRA there is a decrease in the effective arterial volume (EAV) but most nephrons are still functioning, albeit at a low level, with a decrease in GFR but intact tubular function, but very capable of transporting sodium and potassium in the distal nephron. The decrease in the EAV is associated with a decrease in the pressure in the juxta-glomerular (JG) apparatus with an increase in renin and the subsequent increase in both antiogensin and aldosterone. Not only is sodium actively reabsorbed in the proximal convoluted tubule, thick ascending limb of Henle's loop and the distal convoluted tubule, but much of the sodium escaping reabsorption at these sites is taken up in the connecting tubule and cortical collecting duct in exchange for potassium under the influence of the increased levels of aldosterone strongly suggesting intact function of the tubules. As a result there is significantly less sodium delivered into the urine but increased potassium excretion. Therefore, with PRA, the decreasing urine sodium and increasing urine potassium diverge in the urine. In acute tubular necrosis probably in excess of 95% of the nephrons are badly injured and most of the nephrons contribute little to the function of the kidney. These nephrons with injured tubules are incapable of reabsorbing Na and secreting potassium causing a loss of sodium in the urine but minimal potassium secretion. A second reason for impaired sodium reabsorption and potassium secretion could be due to the translocation of Na^-/K^+-ATPase from the basolateral membrane to the cytoplasm, in ATN, which could significantly impair sodium reabsorption in exchange for potassium secretion [17]. So once again sodium and potassium excretion go in opposite directions with a high urine sodium and limited K excretion. Therefore an analysis of the urine Na/K ratio should accurately differentiate these two states.

In an attempt develop a test that fulfilled the prior criteria we looked at more than 200 patients with AKI at the Loma Linda Medical Center and the Redlands Community Hospital over a period of one to two years. These two hospitals were chosen since the investigators were working at these hospitals at the time of study. Twenty-eight of these fulfilled the criteria for ATN (2 proven by renal biopsy) and 42 fulfilled the criteria for PRA. Another 7 patients had PRA that evolved into ATN. We measured the U_{Na}/U_K and U_{Cl}/U_K ratios and compared them to all the previous indices that have been reported.

From Tables I and II it readily becomes apparent that the BUN/creatinine ratios, the urine to plasma creatinine ratios and the U_{Na} are notoriously inaccurate in differentiating between PRA and ATN and therefore should be discarded in the workup of AKI. The FE_{Na} and RFI are much more accurate in diagnosing AKI due to ATN but still have significant overlap in patients with PRA. In 13 of the 42 patients with PRA the RFI was greater than 1 (30%), while in 9 patients the FE_{Na} was greater than 1 (21%). The U_{Na}/U_K ratios were less than 1 in all 42 patients (100%).

The data we have presented on the U_{Na}/U_K show 100% sensitivity and specificity in differentiating PRA from

ATN. In only one patient with ATN where the U_{Na}/U_K ratio was less than 1 (case 1) (Table III) was a patient where the PRA was developing into ATN, and the U_{Na}/U_K ratio was increasing rapidly but was still <1. A larger prospective study should be done on the urinary Na/K ratio, and it may show a lower sensitivity and specificity in the urinary Na/K ratio.

Conclusion

We believe the U_{Na}/U_K ratio after further prospective studies may become the preferred test to differentiate PRA from ATN in AKI for the following reasons: First, it is a much more accurate and reliable test than those presently in use to differentiate between PRA and ATN. It appears to have 100% sensitivity and specificity and therefore may eventually replace all other tests for AKI if it is found to be a more accurate diagnostic test in AKI. Second, it requires only a spot urine specimen for Na and K, and no timed urine collections are required. Third, it does not require drawing any blood samples and does not rely on the appraisal of muscle mass and creatinine production. Fourth, because of its ease of testing, it could be done in poor perfusion states repeatedly to determine if and when the patient may be converting from PRA to ATN so that medication and fluid administration can be adjusted, avoiding fluid overload and CHF and medication overdose. And fifth, it could save considerable costs in the care of patients with AKI.

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Conflict of Interest

None to declare.

Authors' contribution

Theodore Shankel, M.D. (Writing – review & editing)
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Table III. Poor Perfusion ® ATN. The 7 patients in Table III all began their clinical course with a poor perfusion state which subsequently developed into acute tubular necrosis. The same indices depicted are described in Table I. Studies a & b were collected during the poor perfusion phase of their illness, while studies c & d were collected during the acute tubular necrosis phase.

Case #	Serum BUN mgs/dl	Serum Cr mgs/dl	Urine Na/K	Urine Cl/K	FNa	RFI	U/P Creat	BUN Creat
1a	10	0.6	0.12	0.71	.08	.1	191.7	16.7
b	24	1.7	0.2		0.3	.4	62.9	14.1
c	42	3.0	0.74	1.0	1.6	2.21	19	14
2a	31	2.4	0.17	.49	0.2	3.5	45.4	12.9
b	5.3	4.5	1.63	2.95	2.4	29.9	3.8	11.8
d	7.5`	6.2	4.19	3.83	4.1	41.2	2.7	12.1
3a	8.7	3.9	0.62	<0.32	0.6	1.23	22.3	23.6
c	132	5.6	2.04	1.90		5.83	23.6	9.11
4a	59	2.9	0.39	<0.20	0.5	.75	38.6	20.3
c	65	22	2.28	1.81	3.2	7.26	13.5	18.2
d	104	5.7	2.28	1.81	3.2	7.26	13.5	18.2
5a	25	1.7	<0.19	<0.29	0.6	.12	83.5	14.7
b	40	2.8	<0.2	1.1	.27	.31	32.5	14.3
c	89	5.9	5.12	3.62	10.8	17.9	15.08	4.6
6a	49	3.3	0.91	0.81	3.1	4.36	13.3	14.8
c	67	3.8	3.04	3.93	15.8	21.1	17.6	3.7
7a	109	3.4	0.12	0.67	1.16	1.63	7.4	32.1
b	126	4.4	<0.12	0.28	1.03	1.47	6.8	28.6
c	93	4.2	35.7	35	165.9	201.2	0.69	22.1
Mean a & b	56	2.71	0.30	0.54	0.78	1.07	50.44	19.21
S.D.	±37	±1.1	±0.25	±0.28	0.84	1.22	52.55	6.4
Mean c & d	70	4.56	6.38	6.07	6.99	39.82	14.95	13.91
S.D.	±26	±1.32	±10.44	±10.29	6.02	60.39	11.24	7.84

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RESEARCH ARTICLE

The Association between Various Lifestyle Patterns and the Body Mass Index in Adolescents

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Objective: The study aims to analyze obesogenic behavioral patterns of adolescents living in Mureș County, Romania, as well as to establish a relationship between these behaviors and their Body Mass Index (BMI), in an attempt to provide effective prevention strategies for obesity. **Material and Methods**: 153 students between 9th to 12th grade, aged between 14 and 19 years old, from the Vocational and Art Highschool of Târgu Mureș were included in the study. All the candidates filled out an evaluation questionnaire of lifestyle and risky behaviors. The analyzed data were sex, age, residence, BMI and risky eating behavior defined as the consumption of carbohydrates (bread, potatoes, sweets), sodas, junk food, alcohol (wine, distilled beverages, beer), beer separately, level of physical activity (school and extra-school sports activities), sedentary behaviors (≥2 hours/day in front of a screen: personal computer-PC and television-TV), and spending ≥2 hours/day separately on the PC and on the TV. **Results**: A statistically significant association was observed between BMI and consumption of fast-food, tobacco, beer, sedentary behavior and spending ≥2 hours/day in front of the PC. Moreover, there was a statistically significant difference between the BMI values of adolescents presenting all studied risk behaviors compared to those who did not. **Conclusions**: Obesity among adolescents from Mureș County is influenced by lifestyle choices like fast-food, tobacco, beer, sedentary behavior and spending ≥2 hours/day in front of the PC.

Keywords: BMI, adolescents, risk behavior

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Introduction

The global prevalence of obesity almost tripled from 1975 to 2016 [1], as obesity has become a global epidemic even for the young ones: children and teenagers (from 4% in 1976 to 18% în 2016) [2,3]. According to World Health Organization (WHO), in 2016 over 340 million children and teenagers (5 to 19 years) were overweight or obese [1]. Although initially obesity was considered a disease that affected developed countries, after the transformations that occured between 1980-1990, a sudden increase was also manifested in Eastern European countries [4].

Obesity occurs as a nutritional imbalance when energy intake exceeds caloric expenditure. The multifactorial etiology of this disease is dependent on a series of behavioral patterns that are prone to predispose an individual to become obese. Notable examples of obesogenic behaviors include:

Alcohol consumption: according to the WHO [5], 15 years old adolescent drinks 6.3 liters of pure alcohol/year or 13.5 grams of pure alcohol/day.

Fast food intake: the fast-paced lifestyle that characterizes the current society impacts every aspect of human life, including dietary habits. Fast food diet, rich in fat, salt and meat products has slowly replaced the traditional cuisine. Adolescents are often eating fast food, although these aliments fail in providing the daily necessities of vitamins and minerals.

Sweets and Sugar-Sweetened Beverage Consumption (SSBC): children and teenagers with high consumption of sweets and SSBC have an increased risk for developing metabolic syndrome, abdominal obesity, and arterial hypertension [6]. Moreover, SSBC intake has been associated with high triglyceride level, a high waist circumference, as well as high blood pressure [7]. Subsequently, WHO states that a diet high in sugary products has no proven benefits and, as a consequence, reduced free sugars intake is recommended [8].

Smoking: the WHO European region leads in the number of both adult and teenager's smokers (28% and 12%). Although the global sex distribution of smokers showed that 12% of them are male and 11% are female, in Romania 37.4% of the male population smokes, as well as 16.7% of the female population [9]. Smoking is responsible for 7.2 million deaths/year, killing more individuals than acquired immunodeficiency syndrome, malaria, and tuberculosis combined [10].

Physical activity: data from Romania showed that in 2018 only 23% of children and teenagers aged ≤13 years old had a satisfactory level of physical activity, 29% of each were boys and 17% of each were girls. On the other hand,

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30% of teenagers and adults aged ≥ 15 years old reported a proper level of physical activity [11].

Sedentariness (activities like watching TV and spending time in front of a computer): The American Pediatrics Association recommends less than 2 hours/day of entertainment screen time for children and adolescents [12]. Children are exposed to 25000 TV advertisements/ year, approximately 20% of each promoting food and drinks products [13].

A high BMI in childhood and adolescence is associated with a high obesity risk during adulthood, as well as with an increased risk of premature death [14,15]. Also, it has been proven that obesity during childhood and adolescence impacts adult life in various ways, by increasing the cardiometabolic risk (the patients are more prone to develop Diabetes Mellitus, Hypertension, ischemic heart disease or even Stroke) and also by favoring the onset of pathologies like bronchial asthma or polycystic ovary syndrome [16].

Moreover, obesity during youth is associated with various musculoskeletal disorders, psychological conditions and different malignancies (endometrial, breast, ovarian, prostate, liver, biliary bladder, kidney and colon cancer) [1].

The development of a project to evaluate the dimension of risk behaviors in adolescents represents the opening of a sphere of major problems, both in society and in the medical world, namely efficient prevention. Good management is represented by the knowledge of the dimensions of the phenomenon and of the bio-psycho-social particularities, which is why we consider that updating risk behaviors in adolescents is very important. Preventing obesity should remain a priority because the continuous transformation of living conditions predisposes to new risk factors, that the study aims to evaluate the local obesogenic behavioral pattern and to evaluated the relationship between BMI and risk behaviors in adolescents living in Mureş County. Our findings will expand the database and will support future obesity prevention programs knowing the current state of adolescent behavior.

Material and Methods

A cross-sectional study was performed in Mures County, in June 2019, to evaluate the relationship between BMI and the behavioral patterns of adolescents that can lead to obesity. The study was approved by the Ethics Committee of the College of Physicians in Romania and it follows the Helsinki Declaration principles.

153 students from the High School of the Arts, Târgu Mureș, aged between 14 and 19, were asked to anonymously answer a 20 questions questionnaire to identify obesogenic risk behaviors. Each questionnaire was completed in 50 minutes, under similar conditions. Informed consent was presented and granted. The school principal and all participants in the study agreed to the processing and presentation of the data.

Tracked parameters:

- Demographics: gender, age, background
- BMI (kg/m2)
- Dietary habits: Sweets and SSBC consumption, fast-food, bread and potatoes intake
- Alcohol (*wine,* distilled beverages *or beer*) and tabacco consumption
- Physical activity level: school and extra-school sports activities
- Sedentary behaviors: ≥2 hours/day time spent in the front of a screen (television and personal computer), and separately, just in front of a television (TV) and just in front of a personal computer (PC).

The data analysis included descriptive statistics elements (frequency, percentage, confidence interval 95%, mean, median, standard deviation) and inferential statistics. The D'Agostino & Pearson test was applied to determine data normal distribution. For comparison of medians, the Mann-Whitney test, non-parametric test, and Spearman test for correlation determination were applied. The level of significance was set for 0.05. The statistical analysis was performed using the GraphPad Prism 7 utility, the Trial version.

Results

The analyzed group included 11.11% (17, with BMI≤18.4) underweight students, 79.08% (121, BMI=18.5-24.9) students with normal BMI, 8.5% (13, BMI >25.0-29.9) overweight and 1.31% (2, BMI≥30) obese students. The description of the study group in terms of risk behaviors is presented in Table I.

Table II represent the mean ± SD (median) of BMI in adolescents with risk behavior and without it, and p shows if there whether or not a statistically significant difference between the median values of BMI in those with and without consumption.

We found statistical significant positive correlations ($p<0.05$) between the BMI and the excessive hours spent in front of the PC ($r = 0.3494$, CI 95%: 0.1973-0.4850), as well as total time spend in front of the PC and the TV ($r = 0.3023$, CI 95%: 0.1462-0.4437).

Discussions

The results of the current study show that most of the young people in the targeted group have a normal weight, but also a large proportion of the students express obesogenic behaviors. There is a proven correlation between the studied behavioral patterns and the value of BMI.

According to Donna Spruijt-Metz, three major behaviors are influencing obesity: food intake, physical activity and sleep [17]. The findings of the study are consistent with other studies on obesity among young people from a cultural and socioeconomic background that resembles countries like Romania, countries where a rapid transition in dietary habits have been observed, mainly due to

Table I. The description of the studied group in terms of risk behaviors

Parameter studied		Frequency	Percentage	Confidence interval (95%)
Gender	Female	97	63.40%	55.24%-71.03%
	Male	56	36.60%	28.97%-44.76%
Living area	Rural	36	23.53%	17.06%-31.06%
	Urban	117	76.47%	68.94%-82.94%
Daily sweet beverages consumption		74	48.37%	40.22%-56.58%
Sweets intake	Moderate (2-3 days/week)	17	11.11%	6.61%-17.19%
	High (daily)	90	58.82%	50.59%-66.71%
Daily bread and potatoes consumption		127	83.01%	76.10%-88.59%
Fast-food consumption	Moderate (1 days/week - 2 days/month)	66	43.14%	35.17%-51.38%
	High (≥2-3 day/week)	34	22.22%	15.91%-29.64%
Physical activity level	Moderate (≥1 day/week school or extra-school sports activities)	32	20.92%	14.77%-28.22%
	Sustained (≥1 day/week school and ≥1 day/week extra-school sports activities)	115	75.16%	67.54%-81.79%
Smoking habits	Moderate (2-10 cigarettes/day)	60	39.22%	31.43%-47.43%
	Sustained (>10 cigarettes/day)	8	5.23%	2.28%-10.04%
Alcohol intake (≥1 day/week of wine, distilled beverages or beer)		115	75.16%	67.54%-81.79%
Only beer consumption (≥1 day/week)		64	41.83%	33.92%-50.07%
Sedentariness (≥2 hours/day total time spent in the front of a screen)		119	77.78%	70.36-84.09%
PC≥2 hours/day		98	64.05%	55.91%-71.64%
TV ≥2 hours/day		43	28.10%	21.14%-35.93%

Table II. Influence of risk behavior vs risk-free behavior on BMI

Parameter studied	Yes - mean±SD (median)	No - mean±SD (median)	p
Sweets intake	21.43±2.72 (21.20)	21.70±2.99 (21.35)	0.4652
Bread and potatoes consumption	21.54±2.79 (21.20)	21.38±2.86 (21.35)	0.8383
Daily sweet beverages consumption	21.92±2.74 (21.40)	21.13±2.81 (21.00)	0.0855
Fast-food consumption	21.81±2.62 (21.35)	20.95±3.05 (20.10)	0.0382
Physical activity	21.54±2.79 (21.35)	20.88±3.01 (20.55)	0.5888
Smoker	22.05±2.84 (21.60)	21.08±2.70 (20.90)	0.0289
Alcohol intake	21.65±2.95 (21.40)	21.08±2.26 (20.95)	0.3048
Beer consumption	22.51±3.03 (21.95)	20.79±2.38 (20.83)	0.0004
Sedentariness	21.73±2.66 (21.30)	20.74±3.14 (20.15)	0.0367
PC ≥2 hours/day	22.06±2.62 (21.45)	20.53±2.86 (20.20)	0.0008
TV ≥2 hours/day	21.51±3.02 (21.00)	21.51±2.71 (21.30)	0.8407
Risk behavior	22.53±3.20 (22.10)	21.25±2.63 (21.10)	0.0338

urbanization. For example, although most of the Iranian cuisine is made up of bread and rice, bread consumption among Iranian adolescents is 58.4%, lower than that in our study [18].

Another study among adolescents living in Târgu Mureş indicates that 55.30% of teenagers are consuming sweetened drinks [19], a percentage close to the group in our study (48.37%).

In the United States, on the other hand, the trend towards tobacco consumption among adolescents is declining. A study performed for 18 years over students living in Arkansas, USA, shows a decrease in tobacco consumption from 74.4% in 1995 to 52.1% in 2013 [20], possibly due to successfully conducted prevention programs. Moreover, there is a proven association between alcohol intake and tobacco consumption, people who consume alcohol are more likely to smoke [21]. In their study, Akbartabartoori et al found that cigarette smoking negatively affects BMI just in adults over 24 years especially in men, but not in younger people. In women, smoking is associated to central adiposity, in contrast to men witch is not, but a reduction in muscle mass could suggest a lower hip circumference [22]. Interesting, Chiolero et al found that nicotine has two effects: increasing energy expenditure and reducing appetite in the short term, and in contrast, in long term, heavy smokers associate a higher BMI than light smokers or nonsmokers, because of an accumulation of many risky behaviors besides smoking like low physical activity and poor diet that lead to weight gain. Also, smoking is associated with increases insulin resistance and central fat accumulation [23].

In the 2015 ESPAD project, it is reported that almost all Europeans aged 15-16 years old consumed alcohol at least once in their lives (35-96%). The highest consumption rates occur in the Czech Republic, Albania, and Hungary, while the smallest ones are in Iceland, Macedonia, and Norway [24].

According to the results of the current study, there is no statistically significant difference between adolescents who consume alcohol and BMI value, but when the beer consumption was analyzed separately, a relationship between beer consumption and BMI was noticed. Beer has a higher level of carbohydrate as compared to other alcoholic beverages.

There are studies that demonstrate that excessive time spent on the Internet has negative effects on adolescents such as affecting communication and face-to-face interaction with family and friends [25], physical activity [26],

proper eating habits [27], sleeping [28], completing academic tasks [29], also the time spent online is directly proportional to the Internet dependency [30]. According to Griffiths, there are six symptoms presents in pathological Internet users (PIU): salience (more online activities), mood modification (using the Internet as a method of reducing stress), tolerance (need to be more online), withdrawal (increasing the level of depression and irritability in the offline world), conflicts and relapse (failed to disconnect) [31].

Tony Durkee et al classified Internet users into three groups of Internet users: adaptive, maladaptive, and PIU. Results showed a higher prevalence among pathological users compared to the others at risk-behaviors such as poor sleeping habits, tobacco use, physical inactivity and also multiple risk-behaviors was associated with the PIU group (89.9%) [32].

According to a study conducted in Brazil on 6529 teenagers, there is an association between various behaviors like spending time in front of a PC, low levels of physical activity and consumptions of fruits/vegetables and high alcohol intake. Boys living in urban areas are more predisposed to expressing obesogenic behaviors. 21.2% of the total number of participants presented at least one risky behavior, while 37.3% presented two, 28.5% had three, and 8.0% had all the risky behavior mentioned above [33].

Also, the current study states a linkage between BMI and time spend on in front of the PC (≥ 2 hours/day), although there is no direct correlation between BMI and the time spent in front of the TV, probably because entertainment preferences of teenagers have changed over the past years. These results are partially following Roya Kelishadi's study on Iranian teens, where BMI was linked directly to time spent in front of PC/TV (≥ 2) [20].

The study presents a series of limitations. Firstly, there is a relatively small number of participants in the study, all of them being students in the same high school, therefore the data could not be extrapolated and be representative for a region. Because the material and methods of the study were based on a questionnaire, there was also noted a bias of subjectivity and accuracy from the participants.

Another limitation consists in the design of the study. Taking into consideration that the design is cross-sectional, it only offers one snapshot of the current risky behaviors among teenagers, and therefore it cannot predict, follow up and evaluate the consequences in the long term. In the future, the study will extend to a larger population and the study design will be converted so that the data could be representative.

Constant re-evaluation of data regarding the teenager's lifestyle is a useful tool in a modern society where health and quality of life are fundamental for a long prosper life. The emergence of new risk factors for obesity, as well as the continuous transformation of living conditions among people everywhere, explains the need for adequate prevention campaigns against obesity. It is necesary to elaborate more efficient community intervention in schools, to insure the informations about the risks of obesity and ways to reduce it.

Conclusions

There is an association between BMI and the teenagers' predisposition for fast food, cigarettes, beer consumption, sedentary behavior and spending ≥2 hours/day in front of a PC. There is a statistically significant difference between the BMI of adolescents presenting all risk behaviors studied compared with those who do not have them.

Authors'contribution

Irina-Bianca Kosovski, M.D. (Conceptualization; Data curation; Investigation; Resources; Writing – original draft)
Dana-Valentina Ghiga, Lecturer, PhD, M.D. (Methodology; Supervision; Validation; Writing – review & editing)
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Anca Bacârea, Associate Professor, PhD, M.D. (Conceptualization; Project administration; Supervision; Validation; Visualization; Writing – review & editing)

Conflict of interest

None to declare.

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CASE REPORT

Silent Ischemic Stroke Was Revealed after Screening for Cognitive Dysfunction in a Hypertensive Patient with New Onset Atrial Fibrillation – Case Report

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Introduction: Hypertension is one of the most important modifiable risk factor related to cognitive decline and dementia. However, screening for cognitive dysfunction is not part of the routine clinical assessment. **Case presentation**: In this report, we present the case of a 75 year old hypertensive male patient with new-onset atrial fibrillation, admitted to the Cardiovascular Rehabilitation Clinic Târgu Mureș. Apart from the routine clinical assessment, the evaluation of cognitive functions was performed with three different screening instruments which identified cognitive dysfunction. Depressive state was assessed with the shortened 13 items form of the Beck Depression Inventory BDI-13 (BDI-13) and it showed moderate depression which could influence the results of cognitive tests. Detection of cognitive impairment was followed by magnetic resonance imaging, which revealed not only hypertension specific microvascular impairment but also a sequelae of a former stroke in the territory of the left middle cerebral artery and a possible meningioma. **Conclusion**: Screening for cognitive dysfunction in high-risk hypertensive patients can be easily performed and in several cases like ours, can unmask silent cerebrovascular pathologies, leading to prognostic and therapeutic consequences.

Keywords: atrial fibrillation; silent stroke, dementia

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Introduction

There are increasing numbers of patients presenting cognitive decline due to ageing population. The prevalence of mild cognitive impairment is estimated to be from 3% to 42% over the age of 65 years. There is strong evidence that those with mild cognitive impairment have a greater risk to develop dementia than people without cognitive dysfunction [1]mild cognitive impairment (MCI. The hypothesis that chronic arterial hypertension may contribute to cognitive decline [1]mild cognitive impairment (MCI, has been supported by several notorious studies [2–5]. Cognitive dysfunction can be assessed by several easily manageable tests. Population-based screening is not justified, however it is recommended in high-risk groups [6].

In the present report, we present the case of an elderly hypertensive patient with common symptoms, who underwent detailed investigations, leading us to a more complex diagnosis. This had implications on the prognosis and treatment of our patient.

Case presentation

In this report, we present the case of a 75 year old male patient, retired driver, known for approximately 15 years with relatively well controlled arterial hypertension. At the time of admission, he was recently diagnosed with atrial fibrillation (time of onset unknown) and consequently he was admitted to the Cardiovascular Rehabilitation Clinic for cardiovascular evaluation and initiation of anticoagulation therapy. On presentation, he complained of palpitation, dyspnea on effort and memory loss. He was a non-smoker, obese, casual alcohol consumer. The patient has previously been diagnosed with hypertensive cardiopathy with good systolic left ventricular function, left anterior fascicular block, ventricular extrasystole, mild mitral, aortic and tricuspid valve insufficiency and gonarthrosis. No family history for cardiovascular or cerebrovascular diseases, dementia or other forms of cognitive dysfunction were reported.

Findings on general physical examination were unremarkable, excepting a higher BMI of 30.66 kg/m^2. Heart sounds were irregular, without murmurs, heart rate was 82 bpm with pulse deficit of 15 bpm, blood pressure was 130/76 mmHg, oxygen saturation 98% on ambiental air. Laboratory investigations showed normal hemogram, blood lipids (total cholesterol 174.8 mg/dl on statin therapy), uric acid, liver function and normal fasting glucose level, but with impaired glucose tolerance on oral glucose tolerance test. Serum thyroid hormones were also in normal range. No microalbuminuria was detected, however renal function was found slightly decreased (eGFR 68.31ml/min/1.73m2). According to guidelines for the management of arterial hypertension, we evaluated target organ damage [7]. Resting ECG showed atrial fibrillation,

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with 65 bpm average rate, intermediate QRS axis, left anterior fascicular block and no significant repolarization abnormalities. Echocardiography revealed concentric left ventricular hypertrophy (dIVS 14.8 mm, dPW 14 mm) with good systolic function (LVEF 57%), multiple , mild (mitral, aortic) degenerative valve disorders and dilated left atria (LA 54.6 cm^2). Ambulatory blood pressure monitoring was performed under antihypertensive treatment (ACE inhibitor, beta-blocker with vasodilator action), which showed overall mean BP 130/76 mmHg, daytime mean 117/68 mmHg, nighttime mean 137/81 mmHg and reverse dipping pattern (diurnal index -17/-18%). 24 hour Holter monitoring demonstrated atrial fibrillation during the whole monitoring period with a mean heart rate of 70 bpm and ectopic ventricular beats (<1%), no complex arrhythmias, no ST segment deviation, no long RR intervals were found. No significant atherosclerotic plaques were revealed on carotid artery ultrasound (carotid artery IMT 0.83 mm bilateral). Abdominal ultrasound was normal excepting prostate hyperplasia. Eye examination included retinal microvasculature evaluation. Retinal photography revealed vascular nipping with no further signs of vascular changes due to arterial hypertension. Consequently, using the Wong and Mitchell classification, the patient was diagnosed stage I hypertensive retinopathy. Further modifications were seen in the macular area of the right eye, consisting of pigmentary changes of unknown aetiology, localised at the outer retina - choroidocapillary junction.

Apart from the routine clinical assessment, according to the guideline for the management of arterial hypertension, the evaluation of cognitive function was also performed [7]. We selected three well-accepted cognitive assessment batteries: Mini Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA) and the General Practitioner Assessment of Cognition (GPCOG). Depression was also tested, as an influencing factor of cognitive functions, with the shortened 13 items form of Beck depression inventory (BDI-13). The MMSE is the most widely used cognitive test, it is considered to be the gold standard in detection of dementia with a maximum of 30 points, the cut-off value is 24 points [8]. Our patient had difficulties in attention and calculation, recall, repetition, and copy parts. He obtained 21 points, equivalent to mild dementia. The MoCA is also a 30 points test. It is used for detecting vascular mild cognitive impairment [8].

The patient got lower points in visuospatial/executive, attention, language, abstraction parts and no point in delayed recall section. Total obtained score was 21 points (Figure 1). The GPCOG is used in primary care, it consists of two parts (1.Patient examination, 2.Informant interview) [8]. Except the time orientation section, he got no points. Totalizing 1 point, the result meant cognitive impairment. However, it was unnecessary, the patient's wife filled in the second part (informant review needed between 5-8 points [8]). The result was controversial, according to that the patient had no significant cognitive impairment. Finally, it turned out the wife was already diagnosed with dementia. According to all used screening instruments, the patient was diagnosed with CD, with the result of 21 points at MMSE, 21 on MOCA, 1 on GPCOG. BDI -13 questionnaire (11 points) demonstrated moderate depression, which could influence our patient's cognitive functions.

In hypertensive patients with cognitive impairment the markers of cerebral microvascular disease (white matter hyperintensities, silent brain infarcts, lacunar infarcts, microbleeds, brain atrophy) could be demonstrated by brain magnetic resonance imaging (MRI), however, due to the costs and accessibility in most cases it is not feasible [7]. Nonetheless, our patient underwent the brain imaging study, which revealed bilateral periventricular white matter hyperintensities, brain atrophy, lacunar infarcts, furthermore a round mass extra-axial location, parietal meningioma on FLAIR MRI sequence (Figure 1; Figure 2). But surprisingly the radiologist also described on FLAIR and T2 MRI a hyperintense lesion, sequelae of a former stroke in the territory of the left middle cerebral artery (Figure 2).

Based upon these findings our patient's SCORE risk category changed from high to very high risk category followed by therapeutic plan: antihypertension treatment was maintained, as BP was on target level, so was beta-blocker, for rhythm control and BP control. Cardioversion was not an option for several reasons: patient's choice, good control of AF symptoms, dilated left atria, unknown AF time of onset. Patient's stroke risk based on CHA_2DS_2-VASc risk score was 5 points, HAS-BLED score 2 points, with clear indication for chronic oral anticoagulation therapy for stroke prevention [9]. Oral anticoagulation therapy with vitamin K antagonist acenocumarol was initiated, with the achievement of therapeutic target (INR 2.14; 2.5), as the patient could not afford non-vitamin K antagonist oral anticoagulants. The patient needed also a good control of risk factors, including dietary changes for weight reduction and control of glycaemia. After discharge, he was refferred to psychiatric evaluation. He was registered in the national dementia program and Memantine was prescribed for dementia treatment.

Discussion

One of the main target organ damages caused by arterial hypertension is the brain. Stroke, transient ischemic attacks, hypertension-mediated functional and structural alterations in the microvasculature contribute to deterioration of cognitive functions [10]most commonly caused by Alzheimer disease or cerebrovascular factors (vascular dementia. High blood pressure is considered one of the most important modifiable risk factor, besides diabetes, dyslipidemia, obesity, cardio-and cerebrovascular diseases [11]. In addition to, chronic arterial hypertension was also associated with vascular dementia, age-related dementia and Alzheimer disease. The pathophysiological process, however it is not fully elucidated, is considered to be dis-

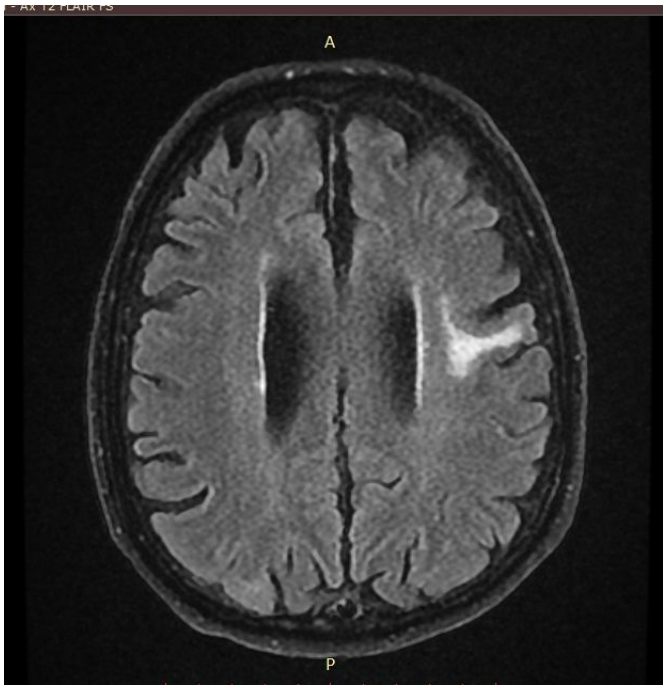

Fig. 1. Bilateral periventricular white matter hyperintensities, significant brain atrophy, hyperintense lesion in the territory of the left middle cerebral artery on FLAIR MRI sequence

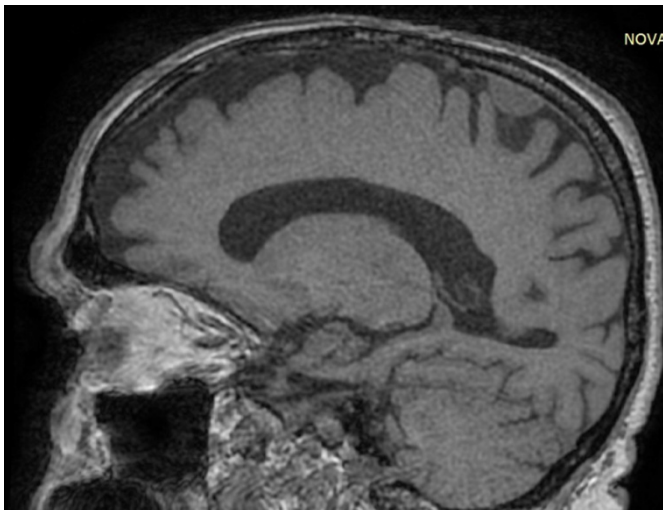

Fig. 2. Cerebral atrophy; round mass extra-axial location, parietal meningioma on FLAIR MRI sequence

ruption of cerebral blood vessels, which activate the process of atherosclerosis, leading to impairment in the cerebrovascular regulatory mechanism. All these alterations lead to cerebral hypoperfusion and structural cerebrovascular changes, like white matter hyperintensities, silent microinfarcts, microbleeds, brain atrophy can be identified with brain magnetic resonance imaging (MRI). Functional changes can be measured by the evaluation of cognitive functions with different tests and questionnaires. Data suggest that the most affected cognitive domains are the executive functions, processing of speed and memory [10] most commonly caused by Alzheimer disease or cerebrovascular factors (vascular dementia. The current guideline for the management of arterial hypertension made a suggestion for the evaluation of cognitive functions as part of the clinical assessment in hypertensive patients with suspicion of cognitive alteration [7].

In the present case, cognitive performances were evaluated owing to the patient's symptoms and as part of a CD screening study amongst all hypertensive inpatients. We identified cognitive dysfunction with three different well-accepted screening batteries. Interpretation of one of the tests (GPCOG) was deeply influenced by the coincidence, that the patient's wife was already diagnosed with dementia. Radioimaging of the brain revealed the markers of microvascular brain disease, which could explain the poor cognitive performance. In addition silent ischemic stroke was also found on MRI. Silent brain infarct is a common incidental finding on brain imaging, it is much more frequent than clinical stroke and is also associated with an increased risk for decline of cognitive performance and dementia [12]. The MRI findings of this case are similar to those abnormalities which can be observed in chronic hypertensive patients. Silent brain infarcts appear more commonly amongst patients with advanced age, arterial hypertension, diabetes mellitus, and smoking [13]. The prevalence of post-stroke dementia is about 30% [14,15], however, it differs in countries and regions being mostly measured by MMSE [16]which varies for the difference between the countries, the races, and the diagnostic criteria. The risk of post-stroke cognitive impairment is related to both the demographic factors like age, education and occupation and vascular factors. The underlying mechanisms of post-stroke cognitive impairment are not known in detail. However, the neuroanatomical lesions caused by the stroke on strategic areas such as the hippocampus and the white matter lesions (WMLs. Overall, this accidental discovery in our case moved our patient to the highest cardiovascular risk category, worsening his prognosis. Furthermore, cognitive evaluation in this patient revealed another complication with important treatment repercussion, which included specific treatment for dementia and changes in target levels regarding his cholesterol.

Although, atrial fibrillation was first documented one week before admission, the presence of dilated left atria suggests the long-standing state of it. The particularity of this case is the presence of atrial fibrillation, which can be an etiologic cause of stroke. Data showed that atrial fibrillation increases the risk of stroke and is associated with deterioration of cognitive functions, hence atrial fibrillation indirectly is related to cognitive impairment. Also there is growing evidence, that atrial fibrillation itself can be a risk factor for worsening of cognitive performance [17]. In the Rotterdam cross-sectional study the prevalence of dementia was doubled among patients with AF compared to controls [18]population-based study in the elderly. Methods Of the 6584 participants in the Rotterdam Study aged 55 to 106 years, detailed information on dementia status and ECG abnormalities was available. Dementia was diagnosed in three phases. First, participants were screened. Screen-positive subjects were tested further. Those with possible

dementia underwent an extensive diagnostic workup. Dementia and dementia subtypes were diagnosed according to prevailing criteria. Cognitive impairment was defined as a Mini-Mental State Examination test score of <26 points for a nondemented subject. Results Atrial fibrillation was diagnosed in 195, dementia in 276, and cognitive impairment in 635 subjects. We found significant positive associations of atrial fibrillation with both dementia and impaired cognitive function (age- and sex-adjusted odds ratios, 2.3 [95% confidence interval, 1.4 to 3.7] and 1.7 [95% confidence interval, 1.2 to 2.5].

Anticoagulation therapy can be challenging in patients with cognitive dysfunction. This problem was enhanced by the presence of the spouse's dementia. In this case, new oral anticoagulants would have been a better choice. Unfortunately, our patient could not afford this, due to low family income. For this patient would have been important if the insurance system supported the cost of a new oral anticoagulants. Still, our patient needs a closer follow-up by the family physician and cardiologist.

Our case also highlights the importance of early detection of atrial fibrillation. Patients detected with persistent atrial fibrillation may have had several asymptomatic episodes of paroxysmal atrial fibrillation which may have also been complicated with asymptomatic cerebral microembolization, as in our patient. For this reason besides invasive monitoring devices, new approaches for atrial fibrillation detection are needed, as smart watches, mobile phone applications, smart dresses, or wareable fitness trackers [19] .

Overall, we can stipulate that bedside tests like MMSE and MoCA provide an easy screening opportunity in this particular group of patients and can have repercussions in the diagnosis and treatment approach. In the light of all the above mentioned risk factors in the literature in association with cognitive impairment, it makes difficult to determine their independent role in our patient's cognitive deterioration. However, as the population ages, the management of cognitively altered and demented patients is going to be a public issue due to a socioeconomic burden. Prevention would be the most cost-effective method through identification and proper treatment of existing risk factors. Definitely, arterial hypertension is the most investigated risk factor in this field. Emerging evidence proves the phrase of midlife hypertension late life cognitive dysfunction. Data shows that BP control in midlife in patients without cognitive impairment at baseline, may diminish the risk of late life cognitive dysfunction, in particularly, in processing speed and executive function, which are considered to be the most affected cognitive domains by arterial hypertension. Long-term outcome should be maintaining a good quality of life as long as possible.

Conclusion

In the present report, we would like to highlight the importance of cognitive assessment in hypertensive patients. Screening for cognitive dysfunction in high-risk hypertensive patients can be easily performed. It can unmask silent cerebrovascular pathologies, having prognostic and therapeutic consequences.

Authors' contribution

Tünde Pál (Conceptualization; Data curation; Formal analysis; Investigation; Writing –original draft)
Zoltan Preg (Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Supervision; Writing – original draft; Writing – review & editing)
Enikő Nemes-Nagy (Conceptualization; Data curation; Formal analysis; Investigation; Resources; Supervision; Writing – review & editing)
Robert Gabriel Tripon (Conceptualization; Data curation; Formal analysis; Methodology; Writing – review & editing)
Beáta Baróti (Data curation; Formal analysis; Investigation; Methodology; Supervision)
Márta Germán-Salló (Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing – review & editing)

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Conflict of interest

None to declare.

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CASE REPORT

Postoperative Lymphorrhagia- a Possible Complication Following Cephalic Duodenopancreatectomy

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Surgery associated with lymphadenectomy may sometimes result in a lymphorrhagia, which usually resolves spontaneously within a few days, sometimes becoming a refractory complication to the treatment. In the case of large flows, particular attention should be paid to hydro-electrolytic and protein losses. We present the case of a patient with persistent lymphorrhagia after a cephalic duodenopancreatectomy for a pancreatic head tumor. From the 5th postoperative day, the patient had a milky-like secretion on the subhepatic drainage tube. The discharge rate was variable, between 500 and 1500 ml per day, requiring parenteral administration of amino acids, plasma and electrolyte solutions. The postoperative progression was slowly favorable, with the patient discharge on the 25th day following surgery. There are several treatment options for a lymphorrhagia following an extended lymphadenectomy, from intensive parenteral therapy to peritoneal-venous shunt or ligation of the lymphatic vessel responsible for the production of lymphorrhagia. In this case the conservative treatment had a favorable result.

Keywords: lymphorrhagia, cephalic duodenopancreatectomy, rare complication

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Introduction

Lymphorrhagia was defined as lymphatic extravasation, in the peritoneal cavity, of a milky-like liquid, rich in triglycerides (1). Postoperative lymphorrhagia has been reported following abdominal surgery accompanied by extensive lymphadenectomy, for example after duodenopancreatectomy (2). Lymphatic extravasation is the result of lymphatic vessels damage, especially in the case of lymphadenectomy in the hepatoduodenal ligament (3). An increased risk of lymphorrhagia, compared to other abdominal surgeries, was found after lymphadenectomy associated with cephalic duodenopancreatectomy (4). In most cases, postoperative lymphorrhagy are spontaneously reversible within a few days. In the case of persistent lymphorrhage, various treatment modalities have been described, from conservative methods to surgical interventions. In this case report we present the case of a patient with cephalic duodenopancreatectomy, followed by a persistent lymphorrhagia, successfully treated by conservative methods.

Case presentation

A 65-year-old male patient was hospitalized in our clinic with the diagnosis of mechanical jaundice with total bilirubin of 8.71 mg / dl and direct bilirubin of 5.69 mg / dl, the transaminases having elevated values - ALT 144 U / L, AST 76 U / L. Abdominal ultrasound revealed liver steatosis, 93/54 millimeter gall bladder, and some endoluminal millimetric stones. Computed tomography showed a leaky gall bladder with sludge with a moderate dilatation of the intrahepatic bile ducts and the common bile duct was dilated to 18 millimeters, without mentioning the cause of the dilatation. After a proper preoperative preparation, surgical intervention was performed, revealing,intraoperatively, a pancreatic head tumor of about four centimeters, imprecisely defined, of tough consistency. Cephalic duodenopancreatectomy was performed with end-to-side pancreatico-gastric anastomosis in a telescoping fashion and double-purse string, end-to-side hepatico-jejunal anastomosis and end-to-side gastro-jejunal anastomosis, subhepatic drainage. The histopathological examination revealed a moderately differentiated ductal adenocarcinoma which goes beyound the pancreatic capsule, invading the muscular layer of the duodenum without lymph nodes metastasis. The postoperative intestinal transit resumed on the 4th day and the oral nutrition of the patient began. From the 5th day postoperatively, a milky-like secretion appeared on the subhepatic drainage tube, pleading for a lymphorrhagia. The level of amylase in the drained fluid was metered, the level being 18 U / L, thus excluding the suspicion of a fistula in the pancreatic anastomosis. Drainage flow was between 500-1500 milliliters per day. The protein level in the drained liquid was 3.2 g / dl. Parenteral loss compensation, human albumin administration, amino acids, electrolytes have been established. From the 10th day after the onset of lymphorrhagia, the subhepatic drainage tube was intermittently blunted, the patient not presenting any abdominal symptoms. The drainage flow was progressively reduced, so the drainage tube was

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suppressed on the 22nd postoperative day. The patient did not show abdominal symptoms, the ultrasound control not highlighting intraperitoneal fluid collections.

Discussion

Postoperative lymphorrhagia is a rare complication in abdominal surgery. The occurrence of this complication may prolong the duration of hospitalization, due to metabolic disorders, protein losses, and sometimes septic complications (5). Among the risk factors for the occurrence of postoperative lymphorrhagia were dissections in the para-aortic area and early enteral feeding (6). Some studies have suggested the diagnostic criteria for postoperative lymphorrhagia: lack of hemorrhage of the drained fluid, absence of increased levels of amylase and bilirubin, presence of increased levels of triglycerides, and mild or creamy appearance of the fluid (7). Talluri describes abdominal distension as the most common symptom, and the triglyceride level in ascitic fluid over 110 mg / dl as a diagnostic element (8). A review from the literature published by Lv et al showed that the lymphatic leakage is due to the damage of the lymphatic channels during the lymph node dissection (9). In the case presented in this paper, no surgical intervention was needed, the parenteral loss compensation therapy, intermittent obliteration, and then removal of the drainage tube had a favorable result. Although the mechanism of lymphorrhagia has not been clearly described, favorable results have been reported in the treatment of postoperative lymphorrhagia following the use of somatostatin and octreotide (10,11). In a study which included patients with D2 lymphadenectomy for gastric cancer, the treatment of the chylous ascites included intrerruption of oral nutrition, total parenteral nutrition, administration of somatostatin analogues and diuretics, medium chain triglycerides and clamping or removal of the drainage tube (12). In case of persistent lymphorrhagia after total pancreatectomy, Bartoli describes as effective therapy the intermittent obliteration of abdominal drainage (3). Inoue reports the performance of a peritoneal-venous shunt in a refractory lymphorrhagia after a liver resection (13), while other authors have recourse to ligation of the lymphatic fistula, using a dye to highlight it (14). Lv et al has made refference to Shao's study that suggests that the lymphatic leakage is a self-limiting complication which can heal within 2 to 3 weeks without other intervention (15). There are studies suggesting that the surgical approach with the direct ligation of the lymphatic fistula should be a first treatment option for postoperative lymphorrhagia, while others plead for an initially conservative approach, as has been shown in the case presented in this paper and to undergo surgical treatment in the refractory cases.

Conclusion

In the case presented in this paper, intensive parenteral loss compensation treatment, concomitant with intermittent obliteration and then removal of peritoneal drainage, had favorable results.

Authors' contribution

Adrian Tudor (Methodology; Project administration; Writing – original draft)
Marian Botoncea (Conceptualization; Investigation)
Cedric Kwizera (Investigation; Writing – original draft)
Bianca Cornelia Tudor (Formal analysis; Investigation)
Cosmin Nicolescu (Investigation; Methodology)
Călin Molnar (Conceptualization; Supervision; Writing – review & editing)

Conflicts of interest

None to declare.

Informed consent

Our patient provided his written consent to participate in the study. This study was approved by the Ethics Committee.

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