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CUPRINS

CONTENTS

RESISTIN IN ALZHEIMER'S DISEASE – FROM PHYSIOLOGICAL TO PATHOLOGICAL MECHANISMS AND BACK

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Abstract: Resistin is an adipokine of great interest for the medical world, being increasingly associated with multiple inflammatory and non-inflammatory pathologies, including Alzheimer's disease. Originally discovered in mice, the presence of resistin was later demonstrated also in humans, but with some structural and functional differences. In the context of the effervescence of research in recent years, this article aims to explore in detail the association between resistin and neurodegenerative pathology. While in the first part the mechanisms via which this adipokine exerts its role in various cells and tissues are presented, in the second part the correlations between resistin and Alzheimer's disease are discussed. The neuroinflammation hypothesis is the central element that brings the two entities together, but other theories could also explain the roles of resistin in neurodegeneration. Based on pathophysiological data and the preliminary results from human and animal clinical trials, it remains to be seen whether resistin will become a reliable biomarker for early detection of Alzheimer's disease in the near future.

Key words: Alzheimer's disease, resistin, adipokine, biomarker, neuroinflammation

INTRODUCTION

Resistin, a small molecule belonging to the adipokine group [1], is currently receiving increasing attention from researchers, as more studies demonstrated its correlation with numerous inflammatory conditions [2]. First described by Steppan et al., 2001 [3] in mice as adipose-tissue-specific secretory factor (ADSF), further research has shown that resistin is also found in humans, being easily determined in the peripheral circulation [4].

There are, however, significant differences between the resistin found in humans and its counterpart in animals (mainly mice and rats). First, while mouse resistin weighs 11 kDa and consists of 94 amino acids, the human resistin is a 12.5 kDa polypeptide containing 108 amino acids and shares only 59% of the murine counterpart sequence identity [5]. The secretory cell of this adipokine also differs when comparing humans to murine species. Murine resistin is produced by adipocytes, its secretion being upregulated by glucocorticoids, growth hormones, testosterone, and prolactin, while insulin, epinephrine, and somatotropin have inhibitory influence [6]. In humans, there are a multitude of cells capable of producing resistin, from mononuclear cells and macrophages responsible for the production of the most important percentage of circulating resistin, to a myriad of other cells for different organs such as the pituitary gland, hypothalamus, the colonic epithelium, pancreas, spleen, and skeletal muscle [7]. In human adipose tissue, research shows that resident non-adipocyte inflammatory cells are the main secretion site for resistin, contrary to the situation in mice [8]. Finally, it is worth mentioning the different structural conformations of resistin in humans. Two isoforms are more frequently encountered, an oligomer weighting 660 kDa and a trimer with a molecular weight of 45 kDa [9]. These two different assemblies were reported to be more active in humans than in mice [10], and seem to play an important role as pro-inflammatory factors [11], however their biological importance is still to be fully determined.

The growing interest for the better understanding of resistin's physiological roles and its behavior in pathological conditions derived from recent results showing a positive correlation between various inflammatory, immune and autoimmune conditions and resistin levels [12]. Resistin is involved in a myriad of pathological conditions where a chronic inflammatory status was assessed. Among the most studied disorders associated with increased resistin levels we mention obesity [13], metabolic syndrome [14], diabetes mellitus type 2 [15], and atherosclerosis [16]. In recent years, research has expanded, covering additional pathologies such as non-alcoholic fatty liver disease [17], Crohn's disease [18], Alzheimer's disease (AD) [19], and also non-inflammatory pathological processes such as as endothelial dysfunction, thrombosis, and dysfunction of smooth muscle cells [20].

In this context, this review aims to present in the first part

Received 31st of January, 2022. Accepted 14th of April 2022. Address for correspondence: Thomas Gabriel Schreiner, University of Medicine and Pharmacy „Gr. T. Popa", str. Universității, no. 16, 700115, Iași, Romania; Phone: +40744860474, e-mail: schreiner.thomasgabriel@yahoo.com

the biological mechanisms by which resistin exerts its roles in physiological conditions and in various pathologies. Subsequently, starting from the neuroinflammatory theory of AD, the authors highlight the potential role of resistin within the pathogenesis of this neurodegenerative disease. Summing up currently available theoretical knowledge and preliminary results from clinical trials, it remains to be determined if resistin can become a valuable biomarker for AD early detection in the near future.

THE MOLECULAR PATHWAYS AND BIOLOGICAL ROLES OF RESISTIN

Being a relatively recently discovered molecule, only a few of the molecular mechanisms by which resistin exerts its effects in physiological and pathological conditions have been highlighted so far.

Resistin acts on a multitude of cell and tissue types [21], through various, still incompletely elucidated mechanisms. For example, in human macrophages, resistin promotes the production of pro-inflammatory cytokines such as TNF-α, IL-6, IL-12 and alters the expression of cell adhesion molecules [22]. By promoting the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and monocyte chemotactic protein-1 (MCP-1), resistin contributes to chemotaxis and recruitment of leukocytes to inflammation sites [23]. Similar effects are also observed in peripheral blood mononuclear cells and hepatic stellar cells [24]. Another target for this adipokyne is represented by both human and mouse blood vessel wall cells. At this level, under resistin modulation, smooth muscle cell proliferation and endothelial cell dysfunction can be observed [25]. These are also preliminary important steps in inflammatory conditions that sustain immune cells adhesion and infiltration via endothelium.

One debatable fact remains the target receptor for resistin. As detailed below (see Table I), there are complementary, sometimes contradictory result in the literature regarding the cellular receptors via which resistin exerts its functions.

Some research shows that resistin binds to the toll-like receptor 4 (TLR4), subsequently exerting its pro-inflammatory functions [26]. In competition with lipopolysaccharide, via binding to TLR4, resistin finally leads to the production of pro-inflammatory cytokines and interferons, thus launching inflammatory and / or immune responses [27]. There are already studies that have demonstrated the effect of resistin via TLR4 mediated pathway in the induction of hypertension and insulin resistance in mice [28], or in facilitating the metastasis of long adenocarcinoma in humans [29].

Daquinag et al., 2011 suggested that decorin could also act as a resistin receptor in adipose progenitor cells in mice [30]. Further research highlighted the possibility for decorin to be a resistin receptor also in humans, as a study conducted on a Japanese population that demonstrated that specific single nucleotide polymorphisms in the vicinity of the human decorin gene are associated with plasma resistin [31]. In a similar fashion, research on a Finnish cohort suggested that decorin polymorphisms might have biological relevance in human vascular pathophysiology, resistin becoming a potential therapeutic target [32].

Other possible receptors include the adenylyl cyclase-associated protein 1 (CAP1), the insulin growth factor-1 receptor (IGF-1R), and the tyrosine kinase-like orphan receptor-1 (ROR-1). Via CAP1, resistin can promote increasing of intracellular cyclic AMP levels, subsequently enhancing protein kinase A and NF-κB activity, this pathway explaining the production of inflammatory cytokines and the related inflammatory response [33]. Several other intracellular signaling cascades may also be relevant for resistin activity in different cells, among the most studied ones being the pathway related to the L-type voltage sensitive calcium channel [34]. Both the external calcium influx and the activation of phospholipase C lead to the enhancement of intracellular calcium, which might subsequently conduct to oxidative stress and immune cells recruiting, sustaining the chronic inflammation [35][36].

Clinical trials suggested a possible relation between resistin and IGF-1R, making IGF-1R a potential receptor for this adipokine. In a study conducted on humans suffering from rheumatoid arthritis, Boström et al., 2011 revealed that abrogation of resistin synthesis is associated with a reduction in IGF-1R expression at the level of the synovium, suggesting that resistin utilizes the IGF-1R pathway [37]. Another research conducted by Bjersing et al., 2013 showed that physical exercise modulates both resistin and IGF-1R levels in fibromyalgia patients, suggesting that the two molecules might share a similar pathway [38].

Finally, one study conducted on mice demonstrated the role of ROR-1 in the mediation of some of the multiple functions such as adipogenesis and glucose uptake that resistin can exert in 3T3-L1 cells [39]. As recent data suggest that ROR-1 is involved in the proliferation, survival, and metastasis of several cancer cells [40], including malignant B cells in chronic lymphocytic leukemia [41], better understanding of this signaling cascade is of great importance for future drug development.

RESISTIN IN ALZHEIMER'S DISEASE – A POTENTIAL BIOMARKER LINKED TO THE NEUROINFLAMMATORY THEORY

Alzheimer's disease (AD), the most common cause of adult-onset dementia [42], is still lacking an effective therapy, despite numerous clinical trials conducted so far. In this context, researchers are increasingly focusing on the prevention of AD, the detection of reliable biomarkers for the early stages of the disease being essential. Resistin, along other adipokines, seems to be an important molecule for AD pathogenesis, as recent studies suggest [19]. For example, the study conducted by Leung et al., 2015 showed that cerebrospinal fluid resistin was significantly associated with $A\beta_{1-42}$ levels and to cognitive impairment changes in symptomatic AD patients [43]. Similar results were found also in peripheral blood samples, as AD patients associated

higher serum resistin levels compared to the controls [44]. There is, however, a great variability between different cohorts that must be taken into account. One recent study demonstrated the ethnicity-related differences regarding neurodegenerative diseases. Molecular biomarkers such as resistin were elevated in African Americans compared to Caucasians, these results suggesting the influence of genetic and ethnic background on AD [45]. Moreover, as antidementia drugs such as acetylcholinesterase inhibitors seem to lower serum resistin levels [46], this adipokine remains a therapeutic target worth to be studied in future trials.

Several pathophysiological mechanisms may explain the association between resistin and dementia. Inflammation remains a constant event in AD onset and development [47], among other potential mechanisms such as excessive accumulation of amyloid beta in the brain [48], the impact of oxidative stress [49], or the role of the vascular component in neurodegeneration [50]. The neuroinflammatory status found in the earliest stages of AD is characterized by increased levels of cytokines and chemokines, resistin being demonstrated to facilitate the secretion of these proinflammatory factors. Although neuroinflammation has both neurodestructive and neuroprotective effects, in case of AD, activation of microglia and the inflammatory cascade has mainly destructive and toxic effects. Moreover, the association between AD and other chronic inflammatory conditions such as obesity and type 2 diabetes mellitus [51] suggests the involvement of shared cellular and molecular factors, resistin being one possible link. Lastly, the multifaceted connection between diabetes and AD has led many researchers to consider AD as "diabetes of the brain" or "type 3 diabetes" [52]. Amyloid beta toxicity is linked to impaired insulin signaling, the increased insulin resistance forming a vicious cycle that subsequently sustains amyloid plaques pathological aggregation. Clinical data has already showed that high plasma resistin levels portend the insulin resistance-related susceptibility to early cognitive decline [53], the role of resistin and other common factors between the two pathological entities still to be completely explained.

CONCLUSION

Associated in the first place with inflammatory conditions and insulin resistance, it has been demonstrated during recent years that resistin plays also relevant roles in other pathological conditions, including AD. Although the pathophysiological mechanisms are still incompletely understood, according to current knowledge and preliminary results from clinical trials, it seems that resistin can become a valuable biomarker for AD early detection in the near future. Future studies are needed in order to fully determine resistin's pathway and its relevance and AD etiology.

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Table I: Potential receptors for resistin

Potential receptor	Molecular pathway Relevant clinical impact	Reference(s)
Toll-like receptor 4 (TLR4)	• Stimulation of pro-inflammatory cytokines and interferon production • Hypothalamic Inflammation • Insulin Resistance • Hypertension • Metastasis facilitation (lung adenocarcinoma)	Kuzmich et al., 2017 [27] Jiang et al., 2016 [28] Gong et al., 2018 [29]
Decorin	• Receptor in adipose progenitor cells in mice • Decorin polymorphism relevant for human vascular pathologies	Daquinag et al., 2011 [30] Onuma et al., 2013 [31] Kunnas et al., 2016 [32]
Adenylyl cyclase-associated protein 1 (CAP1)	• Cyclic AMP increase - protein kinase A / NF-κB pathway • L-type voltage sensitive calcium channel mediated pathway	Avtanski et al., 2019 [33] Singh et al., 2018 [34]
Insulin growth factor-1 receptor (IGF-1R)	• Clinical impact in rheumatoid arthritis and fibromyalgia diagnosis and treatment monitoring	Boström et al., 2011 [37] Bjersing et al., 2013 [38]
Tyrosine kinase-like orphan receptor-1 (ROR-1)	• Mediation of adipogenesis and glucose via 3T3-L1 mice cells • Clinical impact in chronic lymphocytic leukemia	Sánchez-Solana et al., 2012 [39] Aghebati-Maleki et al., 2017 [41]

REZISTINA ÎN BOALA ALZHEIMER – DE LA MECANISME FIZIOLOGICE LA MECANISME PATOLOGICE ȘI ÎNAPOI

REZUMAT

Rezistina este o adipokină de mare interes pentru lumea medicală, fiind asociată tot mai mult cu multiple patologii inflamatorii și non-inflamatorii, inclusive cu boala Alzheimer. Descoperită inițial la șoareci, prezența rezistinei a fost demonstrată ulterior și la oameni, dar cu unele diferențe structurale și funcționale. În contextul efervescenței cercetărilor din ultimii ani, acest articol își propune să exploreze în detaliu asocierea dintre rezistină și patologia neurodegenerativă. În timp ce în prima parte sunt prezentate mecanismele prin care această adipokină își exercită rolul în diferite celule și țesuturi, în a doua parte sunt discutate corelațiile dintre rezistină și boala Alzheimer. Ipoteza neuroinflamației este elementul central care reunește cele două entități, dar și alte teorii ar putea explica rolurile rezistinei în neurodegenerare. Pe baza datelor fiziopatologice și a rezultatelor preliminare din studiile clinice pe oameni și animale, rămâne de văzut dacă rezistina va deveni un biomarker de încredere pentru detectarea precoce a bolii Alzheimer în viitorul apropiat.

Cuvinte cheie: boala Alzheimer, rezistina, adipokina, biomarker, neuroinflamație

THE PHYSIOLOGY OF CARDIOVASCULAR CHANGES ASSOCIATED WITH COVID-19 INFECTION: A REVIEW

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ABSTRACT
Although the clinical manifestations of zoonotic human COVID-19 are well understood to be respiratory, notable accounts of significant cardiac complications ranging from myocyte biomarker elevation, arrhythmias, and myocardial dysfunction to electrocardiographic abnormalities manifestations are being widely published. A few pathology studies conducted so far on patients that died of COVID-19 revealed that up to 35% of them harboured the novel SARS-CoV2 virus in the middle layer of their hearts, the myocardium. As of now, a precise understanding of the effects of COVID-19 on the heart as well as the cardiovascular system as a whole is yet to be well-established, but existing evidence from preceding human zoonotic viral epidemics such as SARS-CoV and MERS-CoV suggests that viral infections in their category could act as triggers for cardiac abnormalities such as acute coronary syndromes, arrhythmias, and heart failure via direct or indirect mechanisms. This review aims to summarise findings on the influence of the zoonotic COVID-19 on human cardiovascular functions so far.
Keywords: Keywords: Coronavirus, Cardiovascular, ACE_2 receptors, Cytokine storm, Spike protein, COVID-19

INTRODUCTION

A Brief Review of the Human Cardiovascular System
The cardiovascular system or the circulatory system are two intertwining terms used to describe the intriguing circulating conduit in man, the system primarily consists of a cardiac pump, which is famously referred to as the heart, a serving circulatory medium which is the blood, then, the intricately designed vasculature network which functions as the conduit through which the blood flows (1). The heart is situated in the middle mediastinum, posterior and slightly to the left of the sternum (2). The myocardium, a middle layer of the heart is structured with a bulk of cardiac tissues which endows it with the ability to pump blood easily (3). Actions of the conducting medium, known as blood, include transportation of nutrients, oxygen, and electrolytes to cells, removal of harmful waste of metabolic processes, and maintenance of the internal milieu (homeostasis) (4). The intricate vasculature system (an extensive network that comprises a large number of vessels such as arteries, capillaries, and veins) contributes to the circulation of blood throughout the cardiovascular system (5). The distribution of oxygen, carbon dioxide, nutrients, and waste products within the body are the central functions of the cardiovascular system in summary. However, the body never ceases to undergo major and minor changes in the course of its life cycle, an integral role and requirement remain the capability of the cardiovascular system to accommodate and execute vastly increasing body demands (3).

HISTORY AND EVOLUTION OF CORONAVIRUSES

Coronaviruses (CoVs) are viral pathogens that possess single-stranded positive-sense RNA and can both mutate and recombine effectively (6). Reportedly, four groups of coronaviruses (α, β, γ, and δ) have been identified, with only two out of the four groups understood as zoonotic: α-coronaviruses (HCoV-229E and NL63) and β-coronaviruses (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) (7). These zoonotic coronaviruses often are the causes of moderate, self-limiting respiratory infections, accounting for up to15% to 30% of all common colds (8). Other influences of these viruses apart from respiratory infections also include, intestinal disorders, neurological diseases and hepatic disorders (9). Patients with persistent cough, high fever, and shortness of breath followed by acute respiratory distress syndrome (ARDS) caused by an unexplained viral infection were reported in Wuhan (China) towards the end of December 2019. The analysis of viral genomes of five pneumonia patients admitted to hospitals between December 18 and December 29, 2019 revealed the existence of the previously unknown β-coronavirus strain in all of them (10).

The newly discovered βcoronavirus presented an 88 percent homology with the sequences of two bat coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21,

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along with a homology of 79.5% and 50% with SARS-CoV and MERS-CoV respectively (10). The SARS-CoV outbreak started on November 2002 in Guangdong Province, southern China, and was most likely initiated by a zoonotic occurrence in China's wild-animal markets. Further research on wild animals revealed compelling evidence that SARS-CoV could have originated from bats (11). While in June 2012, the MERS-CoV crisis broke out in Saudi Arabia (12). Through close contact, the virus spread from infected dromedary camels, the intermediate host, to humans. MERS-CoV is also thought to have originated in bats and spread to dromedary camels in the distant past (12-13). The World Health Organisation (WHO) as of February 11, 2020, officially named the newly emerging zoonotic virus; COVID-19, an acronym for "Coronavirus Disease-2019". This viral pandemic unlike MERS-CoV and SARS-CoV, is capable of causing lethal pulmonary infections in humans (14). The COVID-19 virion, like other coronaviruses, has a nucleocapsid that houses the viral RNA and phosphorylated N protein (15). The nucleocapsid is encased in phospholipid bilayers and protected by a variety of proteins, including the spike glycoprotein trimer (S) (spike protein, S protein), which is found in all forms of CoV, haemagglutinin esterase (HE), the membrane and envelope proteins (M and E), which are found between the spike (S) proteins in the viral envelope (16).

BASIC MOLECULAR PATHOBIOLOGY OF SARS-COV2 VIRUS

The viral SARS-CoV2 presents from the sub-division, Coronavirinae in the family of Coronaviridae. The virus is relatively microscopic (sizes about 60-140nm in diameter) and the genome is an enveloped single stranded positive-sense ribonucleic acid (+ssRNA), which normally ranges from 26 to 32kbs in length, and considerably larger than any other RNA virus. The newly discovered β-coronavirus takes on a round or elliptic form with pleomorphic abilities, and also unique with a halo or crown like appearance due to the presence of protruding spikes from its surface. A certain study in molecular genetics have convincingly demonstrated that the virus consists of six major open-reading frames (ORFs); a reading frame that consists of a continuous stretch of codons (begins at a start codon and ends at a stop codon) known to other coronaviruses, and a number of other accessory genes (17-18). Around two-thirds of viral RNA is translated into two significant polyproteins by the first ORF (ORF1a/b) (19), while the remaining one-third of the viral genome is associated with the other open reading frames of SARS-CoV2, and encodes for four major structural proteins: a spike glycoprotein (S), an envelope protein (E), a nucleocapsid protein (N) and a membrane protein (M) along with various alternative proteins with unknown roles and are not involved in the replication of the virus (20).

The spike protein mediates virus attachment to the host cell and can uniquely recognize the human angiotensin-converting enzyme 2 (ACE2) receptor on the host cell surface (21- 23). In coronaviruses, the envelope protein is a small integral membrane protein which can oligomerize and form an ion channel (24). The E protein has been shown to play a number of roles in the viral replication cycle, including viral assembly (25), virion release (26), and viral pathogenesis (27). The coronavirus nucleocapsid (N) protein is a structural protein that binds directly to viral RNA and provides stability (28). The coronavirus membrane (M) protein is an integral membrane protein that plays a significant role in viral assembly and has been shown to have the ability to cause apotosis. In order to encapsidate the RNA genome, the protein also interacts with the nucleocapsid (N) protein. (29-31).

While SARS-CoV2 contains four structural proteins (S, E, M, and N), it also consists of sixteen nonstructural proteins (nsp1−16). Nsp1 is a leader protein that plays a role in RNA processing and replication. Nsp2 influences the host cell's survival signaling pathway by binding to two host proteins (PHB1) prohibitin-1 and (PHB2) prohibitin-2. Nsp3, a papain-like proteinase protein is the largest encoded by coronaviruses and thought to be involved in the separation of the translated protein. Nsp4 actively modifies ER membranes. Interaction between Nsp3 and Nsp4 is important for viral replication and both consists of transmembrane domains. During replication, Nsp5 participates in the polyprotein process. Nsp6 is a likely transmembrane domain. The combination of Nsp12 and template-primer RNA was significantly increased when Nsp7 and Nsp8 were present. Nsp8 is a peptide cofactor that forms a heterodimer with NSP7 (the other peptide cofactor) and complexes with Nsp12. An Nsp8 monomer unit also complexes with Nsp12 to form the RNA polymerase complex, in addition to the Nsp7-Nsp8 heterodimer. Nsp9 is a protein that binds to ssRNA. Nsp10 is needed for viral mRNA cap methylation. The function of Nsp11is currently unknown. Nsp11 is made of thirteen amino acids and the first nine amino acids are identical to the first nine in Nsp12. The RNA dependent RNA polymerase (RdRp), which is a critical component of coronavirus replication and transcription, is found in Nsp12. Nsp13 binds to ATP and participates in the replication and transcription processes via its zinc-binding domain. The Nsp14 has 3'-5' exoribonuclease activity and N7-methyltransferase activity which is common to coronaviruses. Mn (2+)-dependent endoribonuclease activity is found in Nsp15. Nsp16 is a 2'-Oribose methyltransferase (32-36).

NSP16 binds to the mRNA recognition domains of the U1 and U2 snRNAs to inhibit mRNA splicing after SARSCoV-2 infection. Nsp1 binds to 18S ribosomal RNA in the ribosome's mRNA entry channel, interfering with mRNA translation. Nsp8 and Nsp9 bind to the 7SL RNA, which is found at the Signal Recognition Particle, causing protein trafficking to the cell membrane to be disrupted (37).

THE SPIKE GLYCOPROTEIN, ACE2 RECEPTORS AND TMRSS2 CONTRIBUTIONS IN SARSCOV

The virus binds to a host cell via its target receptor, that is the spike protein (S), which binds to the host cell surface

receptor, Angiotensin converting enzyme 2 (ACE2) as the first step in the SARS-CoV2 entry cascade (38-39). Although ACE2 is widely known to be expressed in the upper airway (goblet and ciliated epithelial cells), alveolar (Type II) epithelial cells of the lungs and pulmonary vasculature. It is also actively present in the heart (cardiofibroblasts, cardiomyocytes, endothelial cells, pericytes, and epicardial adipose cells) and the vascular system (endothelial cells, migratory angiogenic cells, and vascular smooth muscle cells) (40). The enzyme presents as a transmembrane protein (specifically Type 1) with an enzymatic domain located on the extracellular surface of cells where it performs the roles of modifying angiotensin I to angiotensin 1-9 and angiotensin II to angiotensin 1-7 respectively. The resulting angiotensin 1-7 then functions as a counter-regulator of the renin-angiotensin system (RAS) (41-42).

The RAS system regulates both blood pressure and electrolyte balance, and thus plays an important role in cardiovascular physiology (43). ACE2, as a functional receptor for SARS-CoV2 couples with the human Transmembrane Protease Serine 2 (TMPRSS-2), which primes the spike S protein of the virus to facilitate viral entry, hereby significantly increasing chances of viral potency (44-45). Since proteolytic cleavage of the viral S protein is essential for virus binding to ACE2, infection by SARS-CoV2 necessitates coexpression of ACE2 and TMPRSS2 in the same cell type (46). The S protein has been shown to be essential for proper protein folding, modulation of host cell protease substrate accessibility, and antibody binding due to its extensive glycosylation (47-48). Zhou et al concluded from infectivity experiments using Hela cells that expressed ACE2 receptors that ACE2 meditated viral entry into host cells, and that ACE2 could not promote viral entry into cells that couldn't express its corresponding receptor. This discovery without an lingering doubts proved ACE2 as the receptor through which SARS-CoV2 gains entry in host cells (18).

The transmembrane spike glycoprotein (encoded by the structural S gene) forms homotrimers that protrude from the viral surface and has two subunits (S1 and S2) that bind to the receptor. Binding is initiated by subunit S1, and fusion to the infected cell is controlled by a trimeric S2 stalk. The S1 subunit is composed of the N-terminal domain (S1-NTD) and C-terminal domain (S1- CTD) which consists of conserved amino acid residues referred to as RBD (receptor binding domain), while features situated within the S2 subunit are: fusion peptide (FP), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic tail (CT) (23, 49, 18). The S1/S2 protease cleavage site is located on the boundary between the S1 and S2 subunits. The first cleavage (S1/S2 site), known as "priming cleavage," produces S1 (surface) and S2 (transmembrane) functional subunits that remain non-covalently bound in a "prefusion" conformation (50). The human serine protease TMPRSS-2 (Transmembrane Protease Serine 2), a trypsin-like protease that has been shown to cleave monobasic sites and is required for SARS-CoV2 S1/S2 "priming" cleavage between the RBD and fusion peptide (51).

Also, host proteases cleave the spike glycoprotein at the S2 cleavage site (activation cleavage) for all coronaviruses, activating the proteins that are needed to fuse viral membranes and host cells via permanent conformational changes (23, 49). The S1 subunit's receptor binding domain (RBD) directly recognizes the ACE2 receptor (52). The RBM (carboxy-terminal half of the RBD) is the region of the RBD that contains the residues that interact with the host ACE2 receptor (53). The majority of SARS-CoV2 and ACE2 binding sites are found in RBM. The binding site is created by two lobes of RBM and ACE2 and is contained in a peptidase domain at the N-terminus of ACE2. RBM attaches to the ACE2's small lobe on the bottom side. RBM's surface is slightly concave inward to accommodate ACE2 (54).

In an analytical study of a resolution structure of spike protein trimer with a single receptor binding domain in the "up" conformation (receptor accessible), due to this process, the S1 subunit dissociates and the S2 subunit refolds into a stable post fusion conformation, as a result of receptor binding destabilizing the pre-fusion structure. When RBD undergoes conformational transformations, the determinants of the spike protein are hidden or exposed, allowing it to engage a host cell receptor. Two states will hereby result from this process: "down" conformation and "up" conformation. However, SARS-CoV2 couldn't identify the ACE2 on the host cells in the "down" conformation (49). Understanding the SARS-CoV2's receptor recognition mechanism is critical since it dictates the virus's infectivity, host range, and pathogenesis (53, 55).

Cardiovascular Complications associated with SARS-CoV2 Numerous investigations have acknowledged that the viral receptor, ACE2, is also found in a variety of cellular components of the cardiovascular system (i.e., cardiomyocytes, cardiac fibroblasts, pericytes, vascular endothelium, and vascular smooth cells) (56-57). The relationship of the rapidly emerging SARS-CoV2 virus with the cardiovascular system is under research, and it is still being investigated whether the virus will directly proliferate in the heart (58-59), in contrast, the virus is connected to a number of proinflammatory mediators that may have a role in the pathogenesis of cardiac and arrhythmic problems. Myocardial involvement could be caused by direct viral infection, hypoxia-induced apoptosis, or cytokine storm-related cell damage in the body (46). With the knowledge that is currently available, it is also possible to assume that persons with underlying cardiovascular comorbidities are more sensitive to adverse cardiac events after COVID-19 infection (58). According to a study based on single cell RNA sequencing, more than 7.5 percent of cardiomyocytes express ACE2, which could enhance SARS-CoV2 invasion and cause direct cardiotoxicity (60).

ACE2 is a critical regulator of cardiac function in vivo, according to genetic evidence (42), moreover, in humans, circulating ACE2, which is released by endothelial cells, is a

biomarker of hypertension, heart failure, and diabetes, showing enhanced ACE2 activity (59). The activation of the Renin Angiotensin System and the inhibition of ACE2 expression are significant in a number of cardiovascular diseases (56). Severity of COVID-19's health decline has been linked to underlying cardiovascular risk factors and disease, which are thought to be highly correlated with age. COVID-19 is thought to have more catastrophic consequences in older individuals than in younger individuals, with hypertension and diabetes presenting as the two most significant co-morbidities (38). Acute Coronary Syndrome Patients with a suspicion or evidence of acute myocardial ischemia or infarction are referred to as having acute coronary syndrome (ACS). The three conventional kinds of ACS are non-ST-elevation myocardial infarction (NSTEMI), ST-elevation myocardial infarction (STEMI), and unstable angina. In reference to the Fourth Universal Definition of AMI, it can be described as the presence of acute myocardial injury discovered with aberrant cardiac biomarkers in clinical or laboratory evidence of myocardial ischemia in the context of AMI, whether STEMI or NSTEMI (61). Serious viral infections can trigger a systemic inflammatory response syndrome, which raises the risk of plaque rupture and thrombus development, leading to an ST-elevation MI or MI with no ST elevation (62).

In a restricted study of 75 patients hospitalized with SARS, a predecessor of the viral SARS-CoV2, acute myocardial infarction (AMI) caused deaths in 2 of 5 fatal cases (63). Although there is still minute scientific evidence linking COVID-19 to the development of ACS, multiple investigations have found a temporary but significant link between lower respiratory tract infections and acute coronary syndromes, implying serious clinical implications again for SARS-CoV2 virus (46, 64). High expression of ACE2 in pericytes might lead to an onset of microvascular dysfunction, (65) explaining greater propensity for acute coronary syndromes (46). There have been reports of coronary artery involvement and ischemia, including ST-elevation and non-ST-elevation myocardial infarction (STEMI & NSTEMI), with numerous theories explaining coronary artery involvement. Direct viral infection can cause plaque instability and type 1 MI because ACE2 is expressed in vascular endothelial cells. The significant systemic inflammatory response in the third phase of the disease can also cause plaque instability and rupture. COVID-19 can also cause type II MI by causing demand ischemia as a person becomes ill. Hypoxemia coupled with increased cardiac demand owing to systemic infection causes Type-II AMI, which is explained by a myocardial oxygen demand that is disproportionate to the supply (61).

CARDIAC ARRHYTHMIC RISK

Cardiac arrhythmias identified as irregular rhythms of heart such as tachyarrhythmia and bradyarrhythmia are other cardiovascular complications that occur frequently in patients with COVID-19 (66). Arrhythmias in COVID-19 patients can take several forms, including atrial-ventricular block, atrial fibrillation, polymorphic ventricular tachycardia, and complete heart block presenting no escape rhythm (67). Early indications of Arrhythmia might be the first presentation of COVID-19, and new-onset and/or progressive arrhythmia could indicate cardiac involvement. According to a study presenting 137 infected subjects from Wuhan, 7.3% of the subjects had exhibited palpitations as one of their early symptoms for COVID-19 (68). Arrhythmias, such as atrial fibrillation, are also more common in COVID-19 cardiomyopathy, as inflammation is a substrate for atrial arrhythmias (69). Cardiac arrhythmias may be attributed to direct myocardial injury or secondary to systemic alterations due to acute viremia (e.g., metabolic derangement, hypoxia, neurohormonal changes, and inflammatory stress). Hypercytokinemia and arrhythmogenic potential are seen in COVID-19 subjects who are in hyperinflammatory condition. Cytokine storm and rising levels of Interleukin-1, Interleukin-2, Interleukin-6, monocyte chemoattractant protein, and tumor necrosis factor-alpha (TNF-) are also linked to deadly arrhythmias. Atrioventricular block and ST segment elevation have been described as electrocardiogram findings of cardiac arrhythmias. Malignant arrhythmias, such as multifocal ventricular tachycardia/ventricular fibrillation, were observed to develop during COVID-19 and were also linked to increased Troponin T levels (70).

MYOCARDITIS

Although case reports of myocarditis, explained as inflammation of the myocardium exist in COVID-19 infected individuals, it is unknown whether myocarditis is caused directly by the virus or as a result of an inflammatory reaction. Several examples of severe myocarditis with impaired systolic function have been documented following SARS-CoV2 infection (71). Xu et al. discovered sparse levels of mononuclear inflammatory infiltrates in the interstitial space of a COVID-19 patient's myocardium, implying that the new coronavirus may promote myocarditis (72). SARS-CoV2 may share the same pathophysiological mechanism, with direct cardiac muscle injury contributing to myocarditis in COVID-19 infected people. Increased cardiac biomarkers in a significant proportion of individuals have revealed cardiac muscle involvement. An increase in cardiac markers may not be entirely attributed to myocarditis, elevated levels of troponin and brain natriuretic peptide however, are correlated with worse outcomes (73). Acute cardiac injury caused by SARS-CoV2 infection can sometimes lead to fulminant myocarditis, a remarkable clinical crisis with hemodynamic compromise and significant fatality rates ranging from 40 - 70%. Fulminant myocarditis is distinguished by rapid and diffuse cardiac inflammation, necrosis, and eventual ventricular dysfunction, which results in cardiogenic shock, pathological arrhythmias, multi - organ failure, and death (74).

THROMBOEMBOLIC EVENTS

Hypercoagulability has previously been linked to an increased risk of blood clot formation in individuals infected

with SARS and MERS virus respectively (75). COVID19 has been linked to a hypercoagulable state as well. Endothelial cell dysfunction caused by infection results in excessive thrombin production and fibrinolysis shutdown. The hypoxia associated with significant COVID-19 can potentially promote thrombosis by increasing blood viscosity. Increased blood coagulability is also aided by a hypoxiainducible transcription factor-dependent signaling cascade (76). Individuals who did not survive COVID-19 had considerably greater levels of D-dimer and other fibrin degradation products (FDP), as well as longer prothrombin and activated partial thromboplastin durations, according to primary studies on COVID-19 (77). Individuals with COVID19 have been reported to suffer coagulopathy, microvascular, and macrovascular thrombosis (78). Numerous case reports of thrombosis involving a diverse array of vascular beds have now been published. Lower extremity arterial and venous vessels, abdominal veins, bypass grafts, coronary arteries, cerebral veins, pulmonary arteries, large vessel cerebral and carotid arteries, and small distal arteries with vaso-occlusive inflammatory clots have all been explained as having thrombus formation causing a chilblains-like presentation (79).

HEART FAILURE

Heart failure is a leading cause of death in people plagued by COVID-19. It is caused by a variety of myocardial aggression mechanisms, including direct viral myocardial injury, indirect and direct inflammatory damage, O2 supply–demand imbalance, and an increase in atherothrombotic events due to inflammatory destabilization of atheromatous plaques, all of which gives an end result as acute myocardial dysfunction (70-71). Heart failure in individuals with COVID-19 can range from mild heart failure with conserved ejection fraction in the earliest stages of the infection to severe end-stage heart failure and cardiogenic shock with substantial death rates (80).

CONCLUSION

Although the clinical manifestations of zoonotic human COVID-19 are well understood to be basically respiratory, notable accounts of significant cardiac complications ranging from myocyte biomarker elevation, arrhythmias, and myocardial dysfunction to electrocardiographic abnormalities manifestations are being widely published. Precise understanding of the effects of COVID-19 on the heart as well as the cardiovascular system in whole is yet to be well-established, but existing evidences from preceding human zoonotic viral epidemics such as SARS-CoV and MERS-CoV suggest that viral infections in their category could act as a trigger for cardiac abnormalities such as acute coronary syndromes, arrhythmias and heart failure via direct or indirect mechanisms. Further studies on these hypothesized mechanisms are strongly required. Further clinical researches are then required to learn more about the impact of COVID-19 on the cardiovascular system.

Conflicts Of Interest

The authors declare that they have no conflicts of interest in regards to this publication.

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FIZIOLOGIA MODIFICĂRILOR CARDIOVASCULARE ASOCIATE CU INFECȚIA CU COVID-19: O REVIZUIRE

REZUMAT

Deși manifestările clinice ale COVID-19 uman zoonotic sunt bine înțelese ca fiind respiratorii, sunt publicate pe scară largă relatări notabile despre complicații cardiace semnificative, de la creșterea biomarkerului miocitelor, aritmii și disfuncție miocardică până la manifestări ale anomaliilor electrocardiografice. Câteva studii de patologie efectuate până acum pe pacienții care au murit din cauza COVID-19 au arătat că până la 35% dintre aceștia găzduiau noul virus SARS-CoV2 în stratul mijlociu al inimii, miocardul. În prezent, o înțelegere precisă a efectelor COVID-19 asupra inimii, precum și asupra sistemului cardiovascular în ansamblu nu a fost încă bine stabilită, dar există dovezi din epidemiile virale zoonotice umane precedente, cum ar fi SARS-CoV și MERS. -CoV sugerează că infecțiile virale din categoria lor ar putea declanșa anomalii cardiace, cum ar fi sindroame coronariene acute, aritmii și insuficiență cardiacă prin mecanisme directe sau indirecte. Această revizuire își propune să rezumă constatările privind influența COVID-19 zoonotic asupra funcțiilor cardiovasculare umane până în prezent.

Cuvinte cheie: Coronavirus, cardiovasculare, receptori ACE_2, furtuna de citokine, proteină Spike, COVID-19

THROMBOTIC EVENTS AND ANTICOAGULATION TARGETS IN COVID-19 INFECTION

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ABSTRACT

Coagulopathy, vascular damage, and thromboembolic events contribute to the severity of COVID-19 disease. The lack of randomized anticoagulant trials in patients with COVID-19 has made the initial approach rather empirical based on background from other similar diseases. Although some observational studies show that prophylactic and therapeutic anticoagulation can decrease mortality and the number of critical cases, others fail to support this finding, suggesting an increased risk of bleeding. Studies show that the vascular endothelium plays a vital role in the procoagulant status in patients with thrombosis infected with the SARS-CoV-2 virus. COVID-19 associated coagulopathy is a unique condition slightly different from other coagulopathies found in various diseases because of its particularities. There is still significant interest in the mechanism of thrombosis and anticoagulation therapy in patients with COVID-19. Clinicians from experienced medical centers test escalating doses of anticoagulants to optimize current standards of care and understand the risk-benefit of bleeding.

Key words: coagulopathy, deep vein thrombosis, pulmonary embolism, anticoagulant therapy, endothelial dysfunction

INTRODUCTION

Coagulopathy, thromboembolic events, and endothelial damage are key elements observed in patients with COVID-19 and contribute decisively to the severity and progression of the disease. Venous thrombotic events, such as deep vein thrombosis or pulmonary embolism, and arterial thromboses, such as myocardial infarction or stroke, are more common in patients hospitalized with COVID-19, with some meta-analyses showing an incidence of up to 23% in the case of venous thromboembolism in patients admitted to intensive care units (ICU) [1]. These elements are added observations detected in microscopic studies that have shown the presence of microvascular thrombosis in the lungs and other organs, which could explain the diffuse evolution of lung lesions and disease aggression [2].

COVID-19 associated coagulopathy is an essential element that characterizes this entity and has prognostic and therapeutic implications. A particular aspect of this pathology is represented by different dynamics of the value of D-dimers compared to other similar pathologies. Some authors suggest that their systematic evaluation has prognostic and therapeutic implications [3]. All these observations concluded that antithrombotic therapy is essential in managing patients with COVID-19. However, the lack of randomized studies made the initial approach rather empirical at the beginning of the pandemic, based on experience from other similar pathologies.

The antithrombotic and the non-anticoagulant properties of unfractionated heparin / low molecular weight heparins (LMWH) led to the hypothesis that they could influence the evolution of the disease. Non-anticoagulant mechanisms such as heparanase inhibition, competitive binding and inhibition of pro-inflammatory factor synthesis,

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inhibition of histones and neutrophil traps [4], and last but not least competitive inhibition of SARS-CoV-2 virus-cell invasion [5], together with the established anticoagulant mechanism, gives heparins an essential role in the management of patients with moderate or severe disease.

Recommendations for thromboprophylaxis and the use of anticoagulant therapy in hospitalized patients with COVID-19 are mainly based on data from similar pathologies. Some observational studies show that both therapeutic and prophylactic anticoagulation decreases mortality and the number of cases requiring intubation (6). In contrast, others fail to support the same evidence, suggesting an increased risk of bleeding and that therapeutic anticoagulation does not affect survival. [7]. This uncertainty has created confusion regarding the optimal anticoagulant treatment, with significant variability in the intensity of anticoagulation observed in most clinical decisions, hospital protocols, and expert recommendations [8,9].

THE ROLE OF THE VASCULAR ENDOTHELIUM

The level of understanding regarding the mechanisms involved in COVID-19 coagulopathy is constantly evolving; current evidence demonstrates a cascading relationship between endothelial dysfunction, inflammatory / immune activation, and coagulation [10]. The SARS-CoV-2 virus enters cells by interacting with the spike protein and the angiotensin two conversion enzyme receptor (ACE-2), a receptor identified in the lung, heart, brain, and endothelial tissue [11]. The result of this interaction will be an increase in the level of angiotensin 2, leading to a procoagulant status explained by the accumulation of bradykinin and many other mechanisms of the renin-angiotensin-aldosterone system (RAAS) involved in thrombosis [12].

Endothelial injury triggered by viral invasion explains diffuse endotheliitis observed in the lungs, heart, kidneys, and intestines, a phenomenon that will underlie immune activation and cytokine storm, thus promoting hypercoagulability and thrombosis [13]. Physiologically, the vascular endothelium possesses a whole series of functions, such as controlling hemostasis, fibrinolysis, inflammation, vascular permeability and oxidative stress, mechanisms severely disturbed in patients with SARS-CoV-2 infection.

The cytokine storm observed in patients with severe disease is thus explained by the transformation of physiological mechanisms into mechanisms generating endothelial injury and thrombosis. Excessive production of cytokines such as IL-1, IL-6, and TNF-α, together with the alteration of counter-regulation mechanisms, underlie this phenomenon. This process will not be limited only to the local endothelial level. The expression will be systemic, generating an imbalance between thrombotic and antithrombotic mechanisms that favor the accumulation of thrombotic material [14].

Another important mechanism is the action of IL-6 on the hepatocyte, which will promote the exaggerated synthesis of fibrinogen, PAI-1, a fibrinolysis inhibitor, and C-reactive protein, thus resulting in alteration fibrinolysis that can be directly correlated with thromboembolic events [15]. Activation of the coagulation cascade by tissue factor, excessive release of von Willebrand factor (vWf) that will promote platelet aggregation, and altered endothelial fibrinolytic mechanisms observed in patients with a severe disease also explain the formation of thrombotic material. Therefore, we observe that the vascular endothelium has an essential role in explaining the pathophysiological mechanisms involved in COVID-19. Moreover, the elements of endothelial dysfunction begin to be the target of innovative therapeutic approaches [14]. Also, the link between coagulation and inflammation, translated by paraclinical elements such as D-dimers, fibrinogen, pro-inflammatory cytokines, will be directly correlated with the disease, managing to bring a much clearer picture in terms of prognosis, evolution, and outcome of therapeutic intervention.

COVID-19 ASSOCIATED COAGULOPATHY

Initial reports from patients with COVID-19 and respiratory failure revealed the presence of disseminated intravascular coagulation (DIC) criteria (according to ISTH criteria). The incidence is much higher in those who died compared to survivors (71% vs. 0.6%) [16]. Other studies have found that DIC incidence is lower in the early stages of the disease, although there are stigmas of coagulation damage, which has led to the conclusion that a distinct form of coagulopathy turns into DIC once the severity of the disease and septic complications occur [17]. COVID-19 associated coagulopathy (CAC) is a distinct, intensely studied entity, different from coagulopathy in other respiratory viral illnesses, sepsis-induced coagulopathy (SIC), or observed in DIC. In CAC, we observe higher values of D-dimers and fibrinogen, but with minimal changes in prothrombin time and platelet count [18]. Also, consumptive coagulopathy, the primary element present in CID, is rarely observed in the early stages of COVID-19.

There is no official definition of CAC, but it would be imperatively needed for research and therapy guidance. Some authors propose a definition that includes confirmation of SARS-CoV-2 virus infection and two of the following four elements:

- A platelet count below 150 × 109 / L
- A marked increase in the value of D-dimers
- Prolongation of prothrombin time or INR
- Documentation of micro or macro thrombosis

If patients meet only one of the four criteria mentioned above and will have one or more of the following: (i) increased fibrinogen, (ii) increased vWf, (iii) the presence of lupus anticoagulant or antiphospholipid antibodies, they will be at high risk of CAC. The definition presented is not an official or unanimously accepted one, but it manages to shed some light on the understanding of this complex entity. Using this definition, only 10% of patients met the CAC criteria at hospitalization, a percentage that increases sharply at the time of transfer to intensive care to over 60% [19].

CAC presents similarities and clinical-biological characteristics common with CID, SIC, hemophagocytic syndrome, antiphospholipid syndrome, or thrombotic microangiopathy, but with specific unique characteristics [17], incompletely defined and elucidated. These elements could underlie a better understanding of the disease and guide the therapeutic approach.

COVID-19 ANTICOAGULATION

Thromboprophylaxis of patients with COVID-19 has been marked by enthusiasm since the onset of the pandemic, which has been based on clinical observations, pathophysiological investigations, and initial epidemiological data [8,9]. The primary concern was that thromboembolic events had an increased incidence despite applying the prophylaxis standards valid until then [20]. These elements have led some experts to recommend the empirical use of high-intensity thromboprophylaxis [21]. The use of intensive anticoagulation involves an increased risk of bleeding, requiring a closer assessment of the alleged benefit, with some guidelines not recommending the empirical use of escalating doses of anticoagulants [8,9].

The significant interest in this topic has led to the global launch of a series of multicenter randomized trials to answer how combining antithrombotic agents or using different doses could optimize current standards of care and understand the risk-benefit elements in terms of bleeding [22]. Most of these studies included anticoagulants with intermediate or therapeutic doses compared with the usual prophylactic doses, antiplatelet, fibrinolytic agents, and multiple other innovative approaches [23].

The RECOVERY study shows that the addition of aspirin has no benefit in reducing mortality. This statement started to clear up aspirin use in patients with COVID-19 [24]. When it comes to anticoagulant therapy, things are not as clear. The optimal doses of anticoagulant agents in thromboprophylaxis of patients with moderate and severe forms of the disease were investigated in several randomized trials, their results being able to bring a certain degree of clarity.

Three randomized trials (REMAP-CAP, ACTIV-4a, ATTACC) comparing the prophylactic dose of anticoagulant (predominantly low molecular weight heparin) versus the therapeutic dose in hospitalized patients showed that the results differed according to the severity of the disease [25,26]. Thus, in non-critical patients, defined as patients who do not require ventilatory or cardiovascular support, the therapeutic dose increases the probability of survival and reduces the use of cardio-respiratory organ support compared to usual thromboprophylaxis [25]. However, a similar benefit was not observed in critical patients admitted to ICU; the initial strategy of using curative doses of anticoagulant did not increase the probability of survival or the number of days without organ support compared to usual pharmacological thromboprophylaxis [26].

Similar data were observed in the HEP-COVID study, finding that the primary efficacy endpoints, represented by venous/arterial thromboembolic events and deaths of any cause, were significantly reduced using therapeutic doses in non-critical patients. However, the same benefit was not observed in ICU patients [27].

Data from the INSPIRATION trial, which compared intermediate-doses (enoxaparin, 1 mg/kg/day) versus the standard prophylactic dose (enoxaparin, 40 mg/day) in ICU patients, failed to show a clear benefit of intermediate-dose use; there is even a trend of increasing the risk of bleeding, which has not reached statistical significance [28].

Direct oral anticoagulants (DOAC) have also been studied in patients hospitalized for COVID-19. The ACTION study compared the use of therapeutic doses of rivaroxaban with standard thromboprophylaxis and failed to demonstrate a clinical benefit. It pointed out that using therapeutic doses of rivaroxaban significantly increased bleeding events, with the authors concluding that DOAC should be avoided in this category of patients unless an explicit indication is present [29].

The results of these trials are promising, but greater public availability and more rigorous validation would be needed. The logical question is whether guidelines and protocols should be changed to include therapeutic anticoagulation in selected categories of patients [30].

At the time of writing, major guidelines for thromboprophylaxis recommend the use of prophylactic doses of anticoagulant in patients hospitalized with COVID-19 who do not have confirmed thromboembolic events. The use of high-intensity anticoagulation is not supported by currently available randomized studies, at least for patients with severe forms of illness admitted to ICU departments [9,31,32].

The use of therapeutic anticoagulation in patients with moderate forms of the disease remains controversial, with some guidelines recommending this

approach based on the results of recent studies [33], while others await more rigorous validation by the international scientific community [31].

CONCLUSION

The complexity of the balance between anticoagulation, thrombosis, and hemorrhagic complications in patients with COVID-19 makes the therapeutic decision very difficult. The unique international collaboration in large platform trials has managed to bring the first quality data to help clinicians optimize anticoagulant treatment for patients with COVID-19. A whole series of studies will be published in the coming period, which will most likely respond to current uncertainties and help clinicians be more efficient in managing patients with COVID-19.

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EVENIMENTELE TROMBOTICE ȘI ȚINTELE DE ANTICOAGULARE ÎN INFECȚIA COVID-19 (NTM)

REZUMAT

Coagulopatia, afectarea vasculară endotelială și evenimentele tromboembolice contribuie la severitatea bolii COVID-19. Lipsa studiilor randomizate cu anticoagulante la pacienții cu COVID-19 a făcut ca abordarea inițială să fie destul de empirică, bazată pe studiul altor boli similare. Deși unele studii observaționale arată că anticoagularea profilactică și terapeutică poate scădea mortalitatea și numărul de cazuri critice, altele nu susțin această idee, sugerând un risc crescut de sângerare. Studiile arată că endoteliul vascular joacă un rol vital în statusul procoagulant al pacienților cu tromboză, infectați cu virusul SARS-CoV-2. Coagulopatia asociată COVID-19 (CAC) este o afecțiune unică, ușor diferită de alte coagulopatii întâlnite în diferite boli, datorită particularităților sale. Există încă un interes semnificativ în studiul mecanismului trombozei și terapiei anticoagulante la pacienții cu COVID-19. Clinicieni din centre medicale cu experiență testează diferite doze de anticoagulante pentru a optimiza standardele actuale de îngrijire și pentru a înțelege relația risc-beneficiu în ceea ce privește sângerarea.

Cuvinte cheie: coagulopatie, tromboză venoasă profundă, embolie pulmonară, terapie anticoagulantă, disfuncție endotelială

THE IMPACT OF LMWH TREATMENT ON IUGR INCIDENCE IN PREGNANCIES WITH INHERITED THROMBOPHILIA

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ABSTRACT

Thrombophilia is defined as a predisposition to the development of thrombosis being associated with an increased tendency to develop venous thromboembolism. Numerous studies have shown an important association between hereditary thrombophilia and intrauterine growth restriction or preeclampsia. There is still controversy regarding the treatment with low molecular weight heparin of pregnant women with hereditary thrombophilia. The main points of contention are the selection of the type of hereditary thrombophilia that requires anticoagulant treatment, the dose and frequency of administration, and the benefits of LMWH anticoagulant treatment in these patients. Our aim was to evaluate the effects of LMWH treatment on one of the most common fetal complications associated with thrombophilia pregnancies, namely intrauterine growth restriction.

Key words : LMWH, thrombophilia, intrauterine growth restriction

INTRODUCTION

Inherited thrombophilias are a group of genetic disorders that interfere with the coagulation cascade increasing the risk for venous thrombosis and thromboembolism. When pregnancy occurs in a woman with hereditary thrombophilia the risk of thrombosis is potentiated by the physiological state of hypercoagulability existing in pregnancy. The most common inherited thrombophilias are [1]: -

Factor V Leiden thrombophilia is the most common inherited form of thrombophilia with a prevalence in the European general population of 3-8% for one copy of the factor V Leiden mutation;

-Moderate protein S deficiency is estimated to affect 1:500 individuals. Severe deficiency is rare and its prevalence is unknown.

-Moderate protein C deficiency affects about 1:500 individuals.

- Prothrombin-related thrombophilia is the second most common genetic form of thrombophilia, occurring in about 1.7-3% of the European general

-Hereditary antithrombin III deficiency has a prevalence of 1:500-5000 in the general population.

-Plasminogen activator inhibitor 1 (PAI-1) polymorphisms are associated with thrombotic disease. Prior studies have shown a possible link with adverse pregnancy outcomes [2].

Many studies have shown different degrees of association between hereditary thrombophilia and various fetal and maternal complications during pregnancy [3] (eg. recurrent pregnancy loss, foetal growth restriction, late miscarriages, stillbirth and preeclampsia). However, there is no consensus on therapeutic standards in addressing hereditary thrombophilia in pregnancy. Also routine screening for thrombophilic mutations is not considered cost-efficient, as their prevalence is relatively low. [4]

It has been long assumed that insufficient uterine, placental, and fetal circulations result in adverse pregnancy outcomes and that those abnormalities can be defined by the use of Doppler ultrasonography. [5,6[. Uteroplacental blood flow decreases in pregnancies that are complicated by hypertension and intrauterine growth restrictions (IUGRs). This decrease is associated with a pathologic condition of spiral arteries, thought to arise during placentation in the first trimester of pregnancy; thus, it might be possible to predict the development of these conditions by assessing uteroplacental blood flow early in pregnancy with color Doppler [7,8]. The result is abnormal

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uteroplacental blood flow, and this has led to the idea of using Doppler assessment of uterine and umbilical arteries velocity waveforms as a method of screening for these antenatal complications. An abnormal test result is represented by an abnormal flow velocity ratio (systolic/diastolic (S/D) ratio), resistance index, pulsatility index or the presence of an early diastolic notch [9].

Intrauterine growth restriction is one of the most redoubtable complications in pregnancy and is the most common indication for induction of labour before term. Intrauterine growth restriction (IUGR) has been defined as the rate of fetal growth that is below normal in light of the growth potential of a specific infant as per the race and gender of the fetus [10]. It has also been described as a deviation from or a reduction in an expected fetal growth pattern and is usually the result of innate reduced growth potential or because of multiple adverse effects on the fetus. The "normal" neonate is the one whose birth weight is between the 10th and 90th percentile as per the gestational age, gender and race with no feature of malnutrition and growth retardation [11,12]. Possible complications of foetuses with intrauterine growth restriction include: perinatal asphyxia, hypothermia, hypoglycemia, and polycythemia.

The choice of patients who would benefit most from anticoagulation during pregnancy is made by comparing the risk of bleeding resulting from the administration of anticoagulant with the risk of thromboembolism in the absence of anticoagulation. Despite this and the lack of controlled trials, there has been increased use of anticoagulants to prevent VTE (venous thromboembolism) and adverse pregnancy outcomes. Although the effects of anticoagulants on the fetus and the mother may be considerable and there are no controlled studies with proven certainty of safe administration, the use of low molecular weight heparine (LMWH) in pregnancy has been steadily increasing [13].

MATERIAL AND METHODS

We conducted a retrospective study at the Craiova County Emergency Clinical Hospital in which we included 60 patients diagnosed with thrombophilia (various forms) and who also gave birth in the hospital maternity ward. Half of them opted for anticoagulant treatment of LMWH (single dose per day s.c.) and half refused anticoagulation after being presented with the potential risks and benefits of LMWH. The exclusion criterion was the presence of a personal history of venous thrombosis All the patients included in the study had been tested for hereditary thrombophilia using the same laboratory. In addition to monitoring the incidence of intrauterine growth restriction, we also monitored flows on the uterine arteries, umbilical arteries and middle cerebral artery.

RESULTS

1. No anticoagulation

The incidence of intrauterine growth restriction was recorded in approximately 50% of the patients without anticoagulation. The mean age of patients in the non-anticoagulant group was 27.5 years. The weight of fetuses with IUGR ranged from 1400 to 2600 grams and they were all born prematurely. The most common variety of thrombophilia in the IUGR group without anticoagulation was homozygous PAI mutation. Over 80% of patients in the group with IUGR and without anticoagulation had a notch on the uterine artery in the first trimester. This change persisted in almost half of these patients in the second trimester. In patients without anticoagulation and with eutrophic fetuses, the most common variety of thrombophilia was the MTHFR C677T gene mutation. Uterine notch in this group in the first trimester was present in approximately 70% of patients but the incidence decreased to below 30% in the second trimester.

2. Anticoagulation

The mean age in the anticoagulant group was 29.4 years. The incidence of IUGR in this group was approximately 25%. The weights of the fetuses with IUGR in this group ranged from 1450 to 2770 grams. Most varieties of thrombophilia in the group with IUGR and anticoagulation were represented by multiple concomitant mutations of MTHFRC677T, MTHFRA1298C and PAI genes. Almost all patients in the IUGR and anticoagulation group had a notch on the uterine artery in the first trimester and persisted at 50% in the second trimester. Similar to the IUGR and no anticoagulation group, concomitant mutations in the MTHFR and PAI genes were present in the IUGR and anticoagulation group. The frequency of notch on the uterine artery in the first trimester and its persistence in the second trimester was relatively similar to the IUGR and anticoagulation subgroup. If we exclude the anticoagulation criterion IUGR is present in approximately 25% of the studied patients

DISCUSSIONS

Although it is a small study group, the differences between anticoagulated and non-anticoagulated patients are significant in terms of IUGR incidence. The literature offers percentages with a very wide margin ranging from 3% to 35% in terms of IUGR incidence in patients with thrombophilia. The evidence is conflicting with respect to the presence and strength of the associations between inherited thrombophilia and pregnancy complications other than VTE. Even if a heritable thrombophilic defect is found in a woman with recurrent miscarriages or late pregnancy complications, no evidence exists to suggest that LMWH or other interventions, such as antiplatelet treatment, will provide benefit, apart from some studies showing a beneficial effect of low-dose aspirin to prevent preeclampsia in women with a history of preeclampsia, regardless of the presence of thrombophilia [13]

If we restrict our search to higher quality studies, there is currently insufficient evidence to support LMWH use in patients at risk for placenta-mediated pregnancy complications. Alternatively, in the

subpopulation of women with prior history of the more severe placenta-mediated complications (early onset preterm preeclampsia, major preeclampsia, small for gestational age (SGA) ≤5th percentile or pregnancy loss >20 weeks), there remains a possible benefit for LMWH to prevent recurrent complications, however this is based on lower quality evidence (single centre, nonregistered trials) and further high quality research is required to explore this higher risk subpopulation of women.[14]

Polymorphisms of the methylene tetrahydrofolate reductase gene (C677T and A1298C) is known to be implicated in the apparition of preeclampsia [15,16]. The role of mutation in the MTHFR gene, as risk factors for intrauterine growth restriction during pregnancy, is not very well known so far; there are some studies but their results are conflicting and ambigous. In our study group IUGR was found mostly in case of association of multiple mutations including the two most frequent MTHFR mutations, C677T and A1298C.

Patients with plasminogen activator inhibitor 1 (PAI-1) mutation were found in several studies to be at higher risk of developing IUGR [17,18]. In our study PAI mutation was present in the majority of cases which developed IUGR and were not helped by the administration of LMWH.

Increased impedance to blood flow in the uterine arteries in pregnancy , as determined by Doppler ultrasound parameters, has been used as a screening method for preeclampsia and the delivery of a SGA neonate, but this screening method is associated with low positive predictive values indicating that most patients with a positive test will not develop the disease [19]. Our study showed the presence and persistence of uterine artery notch in over 2 thirds of the patients which developed IUGR, regardless of LMWH administration.

CONCLUSIONS

Our study shows a 25% incidence of IUGR regardless of anticoagulation and a double percentage of pregnancies with intrauterine growth restriction in patients without LMWH compared to those who received LMWH. It should be noted that the restriction was encountered regardless of anticoagulation in patients with concomitant mutations on several genes (MTHFR, PAI) and that in the case of patients without anticoagulation the most common mutation is homozygosity of the PAI gene.

In view of the above, we believe that additional stratified studies on subvariant mutations and mutation combinations are needed to accurately determine which patients benefit from LMWH administration.

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IMPACTUL TRATAMENTULUI CU HGMM ASUPRA INCIDENTEI RCIU IN SARCINILE CU TROMBOFILIE EREDITARA

REZUMAT

Trombofilia este definită ca o predispoziție la dezvoltarea de tromboză fiind asociată cu o tendință crescută de apariție a tromboembolismului venos. Numeroase studii au arătat o asociere importantă între trombofilia ereditară și restricția de creștere intrauterină sau preeclampsie. Există în continuare controverse în ceea ce privește tratamentul gravidelor cu trombofilie ereditară cu heparine cu greutate moleculară mică. Principalele puncte de discuții contradictorii sunt reprezentate de selecția tipului de trombofilie ereditară care necesită tratament anticoagulant, doza și frecvența administrării precum si beneficiile tratamentului anticoagulant cu HGMM la aceste paciente. Obiectivul nostru a fost să evaluăm efectele tratamentului cu HGMM asupra uneia dintre cele mai frecvente complicații fetale asociate sarcinilor cu trombofilie și anume restricția de creștere intrauterină.

Cuvinte cheie: HGMM, trombofilie, restricție de creștere intrauterină

VASCULAR CHANGES IN THROMBOPHILIA PREGNANCIES. DOES ANTICOAGULATION MAKE A DIFFERENCE?

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ABSTRACT

Vascular and coagulation cascade changes that occur physiologically during pregnancy are well known. In women with hereditary thrombophilia associated with pregnancy, these changes are exacerbated and produce undesirable effects on pregnancy. Although anticoagulant treatment during pregnancy seems to be the logical solution in these cases, however, many studies show net benefits of using anticoagulants only in patients with previous adverse pregnancy outcomes (pregnancy loss, preeclampsia, eclampsia, intrauterine growth restriction). In this study we aimed to observe the effects of HGMM treatment on vascular flow in the uterine arteries, umbilical artery and middle cerebral artery.

Key words: LMWH, thrombophilia, intrauterine growth restriction, Doppler

INTRODUCTION

Doppler ultrasonography has been expanding day by day thanks to its superiority in analyzing hemodynamics. Changes in fetoplacental and uterine hemodynamics can be identified without requiring an invasive procedure on many sites, particularly umbilical artery, fetal middle cerebral artery and uterine artery, for topics such as intrauterine growth retardation, fetal anemia followup and management, preeclampsia and even the prediction of poor obstetric outcomes. [1] Blood flow to the placenta is provided by the uterine artery . Mostly Doppler evaluation of uterine artery is being used for preeclampsia prognostic [2]. By analyzing vascular placental resistence using Doppler ultrasonography on uterine artery, serious aid is given in the management of perinatal moratality from intrauterine growth restriction [3]

Another important parameter in evaluation of palcental perfusion is umbilical artery flow whos degradation is associated with adverse pregnancy outcomes such as IUGR. Abnormal umbilical artery blood flow has been implicated in pregnancy complications and fetal demise. Its relation to histopathological changes in the placenta and to maternal or fetal thrombophilia is not as well understood.[4] Vascular pulsatility index in fetal middle cerebral artery (MCA) is very important for the antenatal follow-up and management of IUGR in terms of the brain sparing effect defined as centralization . It is also very important to predict the poor obstetric outcomes as a component of “cerebro-placental ratio” (CPR) which has been investigated frequently in the recent years. [5]. Thrombophilia represents an important factor in maternal-fetal complications during pregnancy and it is known to increase the risk of adverse pregnancy outcome. According to current data, there is an extensive literature debate regarding the coagulation disorder in pregnant patients, that may lead to various complications and increase the morbidity and mortality of both mother and fetus, and lead to adverse pregnancy outcome, such as spontaneous abortion, intrauterine growth restriction, placental abruption, preeclampsia, stillbirth or venous thromboembolism [6]. Thus an appropriate treatment initiated at the right time can make the difference between a good or bad pregnancy outcome for both mother and offspring. Anticoagulation with low molecular weight heparins (LMWHs) is a well-established antithrombotic practice for primary and secondary thromboprophylaxis during pregnancy. Due to their excellent safety record, LMWHs have been offered to women at high risk of an adverse pregnancy outcome in advance of scientific evidence [7]. The administration of LMWHs in the

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prevention of pre-eclampsia and small for gestational age (SGA) fetuses is based on biological plausibility and extrapolation from antiphospholipid syndrome [8]

MATERIAL AND METHODS

The study is a retrospective one on, patients admitted at the Craiova County Emergency Clinical Hospital. It was conducted on 60 patients with different forms of thrombophilia (various gene mutations) and who were followed until the time of birth. 30 patients received anticoagulant treatment (single dose per day of LMWH s.c.) and 30 decided to refuse anticoagulation after being presented with the potential risks and benefits of LMWH.

The exclusion criterion was the presence of a personal history of venous thrombosis and adverse pregnancy outcomes.

All the patients included in the study had been tested for hereditary thrombophilia using the same laboratory. We searched for the two most common mutations of MTHFR gene, C677T and A1298C and also mutations of the PAI gene. In addition to monitoring the incidence of intrauterine growth restriction, we also monitored flows on the uterine arteries, umbilical arteries and middle cerebral artery in the second and third trimester.

RESULTS

1. No anticoagulation

We had 30 patients who refused to be treated with LMWH.

We observed a natural decrease in uterine artery pulsatility index from second to third trimester in all patients without LMWH. Of the 30 patients without anticoagulation, 7 (representing 23%) had an abnormally high pulsatility index (PI) on the uterine artery in the 2nd trimester and only 2 of these 7 had the same values in the 3rd trimester. IUGR pregnancies occurred in 5 of the 7 patients with abnormally high PI in the 2nd trimester, representing 16% of patients without anticoagulation and 71% of patients without anticoagulation and with high PI in the 2nd trimester.

21 out of 30 patients had 2nd trimester uterine artery notch which persisted only in 10 patients in the 3rd trimester. More than 50% of the patients in this group had a ratio of less than 1 for ACM/Umbilical Artery relation in the 2nd trimester. In the third trimester ACM/Umbilical Artery < 1 was found in over 75% of patients.

2. Anticoagulation

As in the previous group, a natural decrease in the pulsatility index was observed on the uterine artery in the 3rd trimester. High values of PI were found in 23% of this group in the 2nd trimester with only 2 remaining the same in the 3rd trimester. IUGR occurred in 2 (6,6%) out of the 7 patients with abnormally high PI in the 2nd trimester. 26 out of 30 patients had a notch on the uterine artery in the 2nd trimester with only 6 being present in the 3rd trimester. ACM/Umbilical artery ratio was less than 1 in about 35% of the patients in this group in the 2nd trimester growing up to 50% in the 3rd trimester.

DISCUSSIONS

ACM/UA pulsatility index ratio is a very good predictor of adverse outcome in the fetuses of women with preeclampsia and gestational hypertension [9]. Early studies (2003) showed some improvement in umbilical and middle cerebral artery flow under thromboprophylaxis [10]. In our study the anticoagulated patients had a significantly lower percentage of subunit ratio of ACM/Umbilical artery. The use of LMWH to improve uterine artery indices has been studied and proven especially in patients with gestational hypertension [12] and also in non-thrombophilic placental mediated complications [13]. There are some studies that concluded that the use of LMWH in thrombophilic pregnant women do not add any benefits regarding flow indices on uterine arteries. Our study showed no improvement in uterine artery PI with LMWH administration, the percentage of patients with abnormally high PI was the same in both anticoagulated and non-anticoagulated groups. Still, IUGR occurred in a smaller percentage in the anticoagulated group suggesting that uterine arteries flow indices might not be implicated in the mechanism of IUGR emergence.

The effect of LMWH administration on uterine artery notching in patients with thrombophilia is controversial and even when the results show an improvement in blood flow there is no significant effect on pregnancy outcomes[14]. Our study showed only a minor improvement in the presence of uterine artery notching with insignificant results on IUG occurrence crossed with uterine artery notching.

CONCLUSIONS

Administration of LMWH was of little use, no major improvement being found regarding the vascular indices on the uterine artery or umbilical/middle cerebral artery ratio. Uterine artery notch improved from 2nd to 3rd trimester irrespective of anticoagulation. IUGR incidence was significantly lower in the group with anticoagulation altough vascular indices were not improved by LMWH. From our small study group we can conclude that anticoagulation in patients with thrombophilia does reduce the IUGR incidence but not by improving the Doppler indices on uterine artery, umbilical artery or middle cerebral artery. Thus different mechanisms of actions are probably responsible of the poor pregnancy outcomes in women with thrombophilia.

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MODIFICARI VASCULARE IN SARCINILE CU TROMBOFILIE. ANTICOAGULAREA FACE DIFERENTA?

REZUMAT
Sunt binecunoscute modificarile vasculare si ale cascadei coagularii ce apar in mod fiziologic in timpul sarcinii. In cazul femeilor cu trombofilie ereditara asociata sarcinii aceste modificari sunt accentuate si produc efecte nedorite asupra sarcinii. Desi tratamentul anticoagulant pe perioada sarcinii pare sa fie solutia logica in aceste cazuri, totusi, multe studii arata beneficii nete la folosirea anticoagulantelor doar la pacientele cu antecedente obstetricale nefavorabile (pierderi de sarcina, preeclampsie, eclampsie, restrictie de crestere intrauterina). In acest studiu ne-am propus sa observam efectele tratamentului cu HGMM asupra fluxurilor vasculare la nivelul arterelor uterine , arterei ombilicale si arterei cerebrale medii.
Cuvinte cheie: HGMM, trombofilie, restricție de creștere intrauterină, Doppler

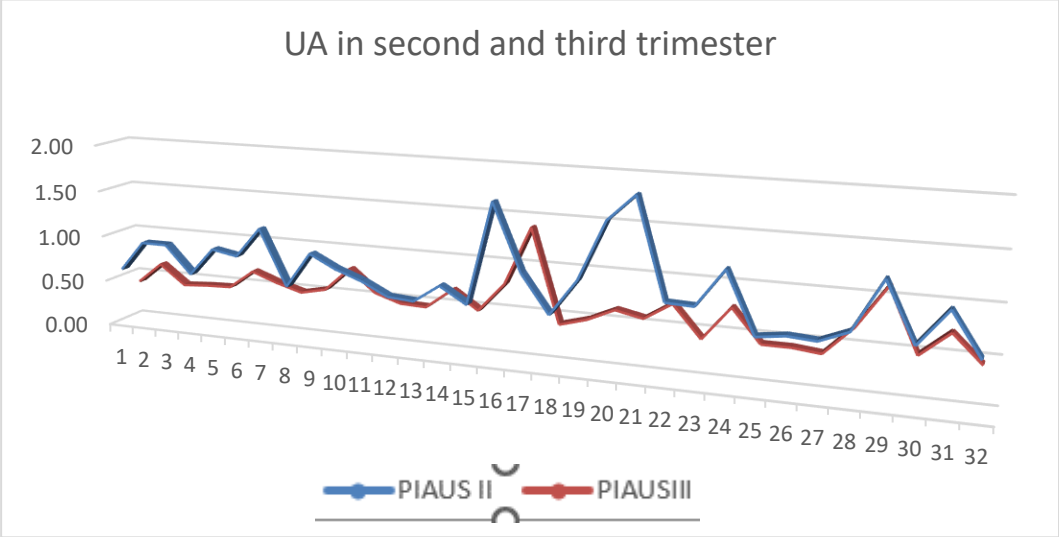

Fig 1. Uterine artery doppler evaluation in second and third trimester in the group without LMWH

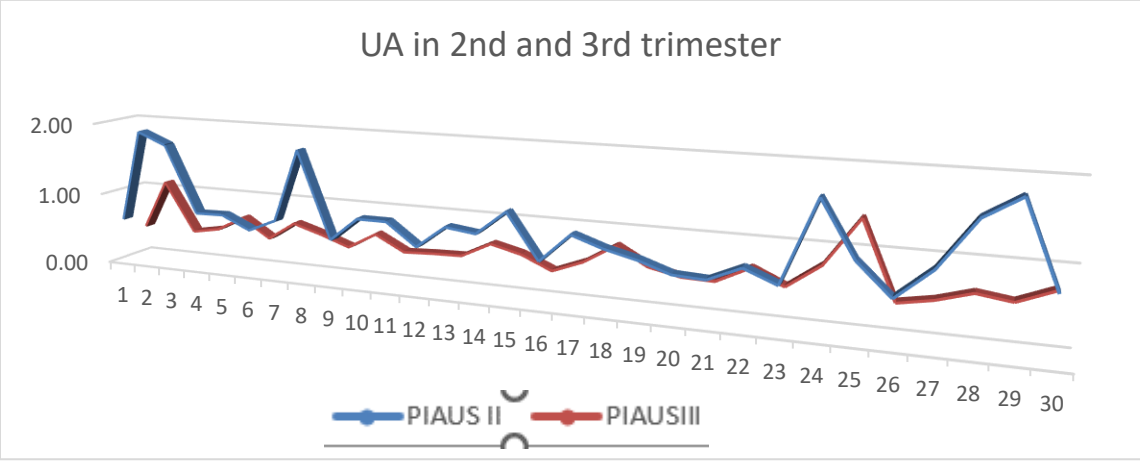

Fig 2. Uterine artery doppler evaluation in 2nd and 3rd trimester in the group which received anticoagulation

CHALLENGES IN THE MANAGEMENT OF INVASIVE BASAL CELL CARCINOMA OF DORSUM NASI

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ABSTRACT

Basal cell carcinoma is the most frequent malignant tumor of the skin that usually appears on the sun exposed areas and macroscopically looks like skin-colored bump with rolled edges or a patch with scaly-waxed surface that doesn't heal with time or local topical treatment.

We present the case of a 71-years old patient that was redirected to plastic surgery department by a dermatology specialist with a presumed diagnosis of squamos cell carcinoma after punch-biopsy and dermatoscopy. The unusual macroscopically appearance pleaded to SCC due to large ulceration borders and center. Images of CT showed no regional adenopathy, but local infiltration of alar-cartilages and superficial part of the septum.

We performed 3 stages excision surgeries with histopathology examination for a radical excision, followed by a 2 stage nose reconstruction with frontal flap based on supraorbital and supratrochlear arteries and it's structure was based on osseous grafts, cartilage grafts recruited from left concha and remaining septum and oral mucosal grafts for nasal cavity lining.

The particularity of this case report is the depth of the tumor invasion, the involvement of the alar cartilages that needed to be excised, the multiple stages of tumor excision in order to obtain free-tumor edges and the multistep reconstruction of the nose in order to obtain a strong and breathable structure.

Keywords: nose reconstruction, basal cell carcinoma, multi stage excision, frontal flap, cartilage grafts

INTRODUCTION

Basal cell carcinoma is the most frequent nonmelanotic skin cances, has a local invasive pattern, but almost never metastasize. In Europe the incidence of BCC (basal cell carcinoma) is 15/100 000 per year, and it had doubled in the last 15 years.[1,2] The estimated numbers of new cases of cancer and deaths in 2021 cannot be compared with other previous years, due to the pandemic. Covid 19 pandemic already creates decreased access and fewer resources for diagnosis and cancer treatments, which will influence a higher mortality, a low survival rate and a lower incidence of new cancer cases that are more invasive and request multi-step approach.[3,4,5,6].

MATERIAL AND METHODS

A 80-year-old man was reffered from a local dermatology clinic to our department in July 2021, for a skin tumor on dorsum nasi. The tumor was 2.5x3.5 cm (fig.1) , with an ulcerated and bleeding surface and it had increased in size in the last 2 weeks with at least 15%.

anticoagulation after being presented with the

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Paraclinical investigations and pathological features

The punch biopsy revealed the suspicion of squamous cell carcinoma with atypical mitosis that invades the basal membrane. Due to SCC suspicion, we recommended CT scans and an oncology consult.

CT scan with iv contrast revealed discrete iodofile modification of dorsum nasi and right paramedian in the middle part of the nose, involvement of the right alar cartilage and anterior portion of septum. No adenopathy. No systemic visible metastasis.

Histopathology

Excision biopsy result revealed an ulcerated tumor proliferation of the skin, organized in cords and spans of hyper chromatic basal cell, with pleomorphic form, close to the epidermis and diffused in the entire dermis, with the invasion of muscular layer and of the perichondrium beneath. Multiple centers of chondromalacia associated with important inflammation and fibrosis. Zones with squamous cell metaplasia and intratumor keratinization. As a conclusion the lesion represents an infiltrating basal cell carcinoma with profound margin infiltrated (fig 2).

Treatment and Prognosis

The tumor was primarily excised with a 5 mm lateral margins and in block with the right alar cartilage, anterior septum, with a partial, medial left alar cartilage and subjacent mucosa for a radical treatment (fig 3) and the defect was covered with a tulle gras dressing until the histopathology result came in (fig 3).

Histopathology result revealed not SCC (squamous cell carcinoma) like previous punch biopsy, but basal cell carcinoma. The profound margin oriented in area 2-4 o'clock in the rapport with the cranial pole of the tumor were tumor positive, which lead to the decision of re-excision in the noted areas.

The second histopathology result showed tumor free margins (fig 4), but in order to maintain a radical surgical treatment we decided that in the same operation to minimally re-excise the border in a 3rd stage (fig 5) and perform the reconstruction of the nose.

We performed frontal flap for external coverage of the dorsum nasi (fig 6), conchal cartilage graft (fig 7) and inferior septum graft (fig 8) and bone graft to reconstruct the alar cartilages and anterior septum, and a oral mucosal graft (fig 9) to reconstruct the nasal cavity lining that was excised.[7,8,9,10,11,12]

After putting in place all the cartilage grafts and suturing to the remaining cartilage (fig. 10), the frontal flap was created and rotated on top of the defect, suturing the mucosal graft on the internal side of the nose (fig. 11), The frontal flap will maintain its pedicle 3-4 weeks (fig. 12) for an appropriate vascular integration and it will be trimmed and divided after that in a second-stage-reconstruction operation.[13,14,15,16,17]

The donor area on the forehead for the frontal flap was primary closed, but was in tension and had suffered, and so a skin graft for the frontal area was planned for the second step surgery, as to pedicle-division and flap trimming (fig. 13).[16,17]

Frontal flap reconstruction of the nose is the gold-standard procedure for large tridimensional nasal defects and with time, the skin and subcutaneous tissue will readjust and give a close to normal aspect of the operated side (fig. 14). Due to reconstruction of the septal and alar cartilage, the nasal cavity is not obstructed and nostrils have a normal shape (fig. 15)[18,19,20,21,22,23]

The patient continued with the oncologist recommendations and the adjuvant treatment (radiotherapy) after the final operation is healed. The forehead flap brings tissue that is very well vascularised into the defect area and is safe for adjuvant radiotherapy.

RESULTS

The patient prognosis is good, after complete and radical excision with reconstruction of nasal lesions and with good follow-up and adjuvant procedures. The patient is very happy with the post operatory aspect and breathes normally with his nose.

DISCUSSIONS

Late diagnosed and invasive skin cancers of complex structures need aggressive treatments and multi-step surgeries in order to obtain a radical excision and a close to normal aspect or function. [24,25]

Punch biopsy result may not be the same as the excision- biopsy due to polymorphic cells within the tumor and interdisciplinary investigation need to be done before deciding the treatment plan. [26,27,28]

Rare cases of mixed tumor histopathology need a thorough investigation, a multi-stage radical treatment and, if necessary, post operatory adjuvant treatment, followed by close follow-up exams in the years to come.[29]

CONCLUSIONS

Our study shows a 25% incidence of IUGR regardless of anticoagulation and a double percentage of pregnancies with intrauterine growth restriction in patients without LMWH compared to those who received LMWH. It should be noted that the restriction was encountered regardless of anticoagulation in patients with concomitant mutations on several genes (MTHFR, PAI) and that in the case of patients without anticoagulation the most common mutation is homozygosity of the PAI gene.

In view of the above, we believe that additional stratified studies on subvariant mutations and mutation combinations are needed to accurately determine which patients benefit from LMWH administration.

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PROVOCĂRI ÎN MANAGEMENTUL CARCINOMULUI BAZOCELULAR INVAZIV AL DORSUM NASI

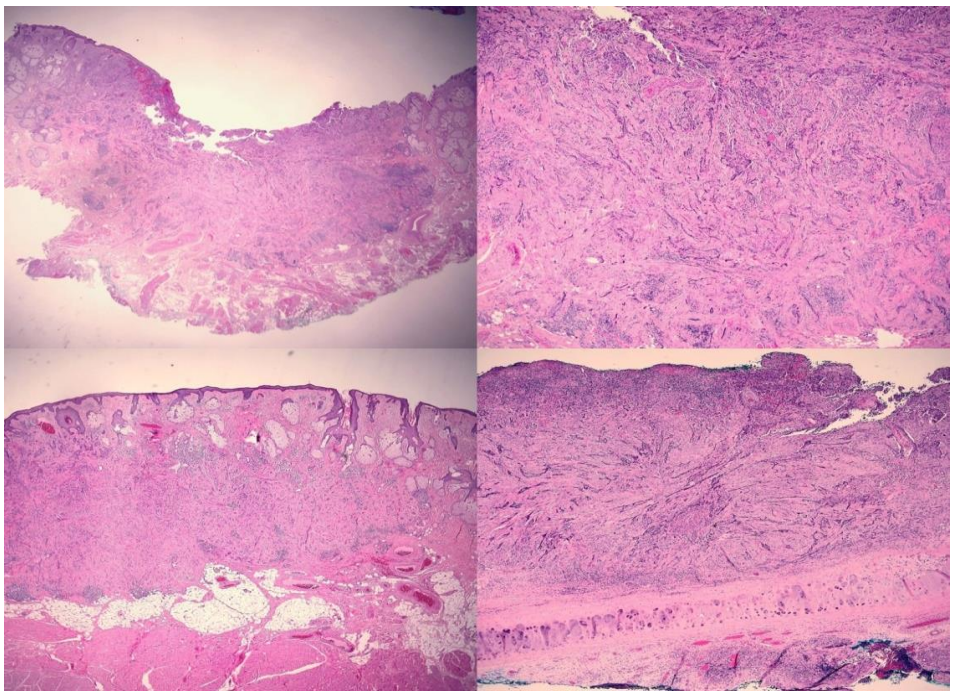
REZUMAT

Carcinomul bazocelular este cea mai frecventă tumoare malignă a pielii, care apare de obicei pe zonele expuse la soare și macroscopic se prezintă ca o umflătură de culoarea pielii cu margini rulate sau un plasture cu suprafață solzoasă ceară care nu se vindecă cu timpul sau tratamentul local local. Prezentăm cazul unui pacient în vârstă de 71 de ani care a fost redirecționat către secția de chirurgie plastică de către un specialist dermatologie cu un presupus diagnostic de carcinom scuamos după punch-biopsie și dermatoscopie. Aspectul macroscopic neobișnuit a pledat pentru SCC din cauza granițelor și centrului ulcerației mari. Imaginile CT nu au arătat adenopatie regională, ci infiltrație locală a cartilajelor alare și a părții superficiale a septului. Am efectuat intervenții chirurgicale de excizie în 3 etape cu examen histopatologic pentru o excizie radicală, urmată de o reconstrucție a nasului în 2 etape cu lambou frontal bazat pe artere supraorbitale și supratrohleare și structura sa a fost bazată pe grefe osoase, grefe de cartilaj recrutate din conca stângă și sept și oral rămas. grefe de mucoase pentru căptușeala cavității nazale. Particularitatea acestui raport de caz este profunzimea invaziei tumorale, implicarea cartilajelor alare care trebuiau excizate, etapele multiple de excizie a tumorii pentru a obține margini tumorale libere și reconstrucția în mai multe etape a nasului pentru a obține o structură puternică și respirabilă.

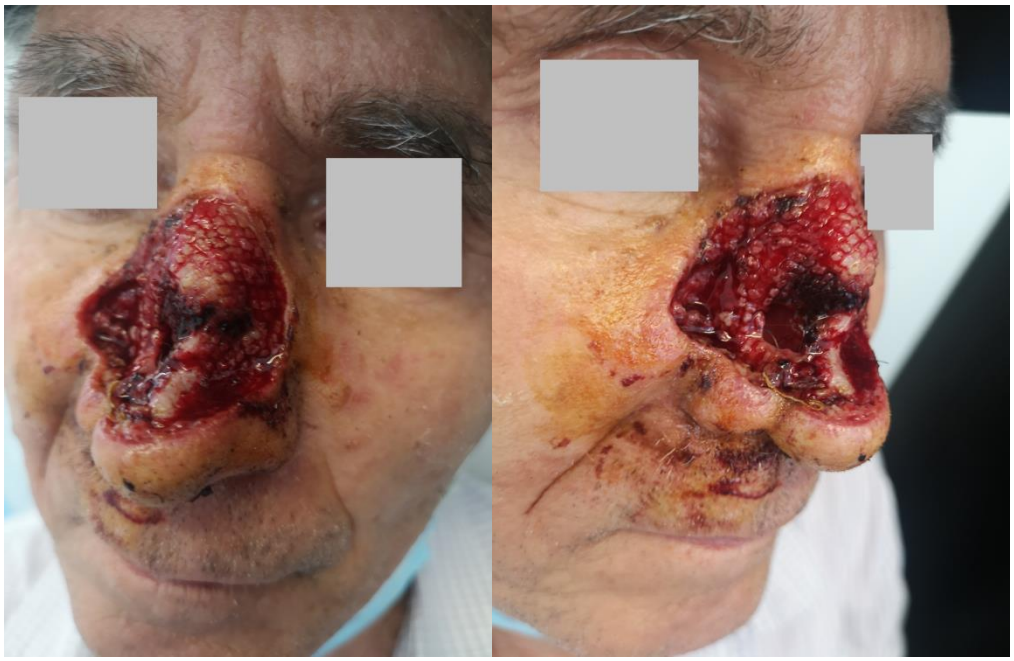
Cuvinte cheie: reconstrucție a nasului, carcinom bazocelular, excizie în mai multe etape, lambou frontal, grefe de cartilaj

FIGURES

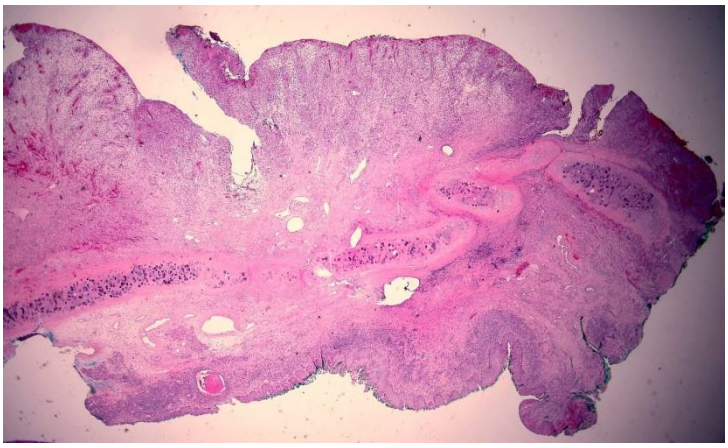
(fig1. Initial evaluation of tumor. Ulcerated and bleeding surface invading the beneath structures)



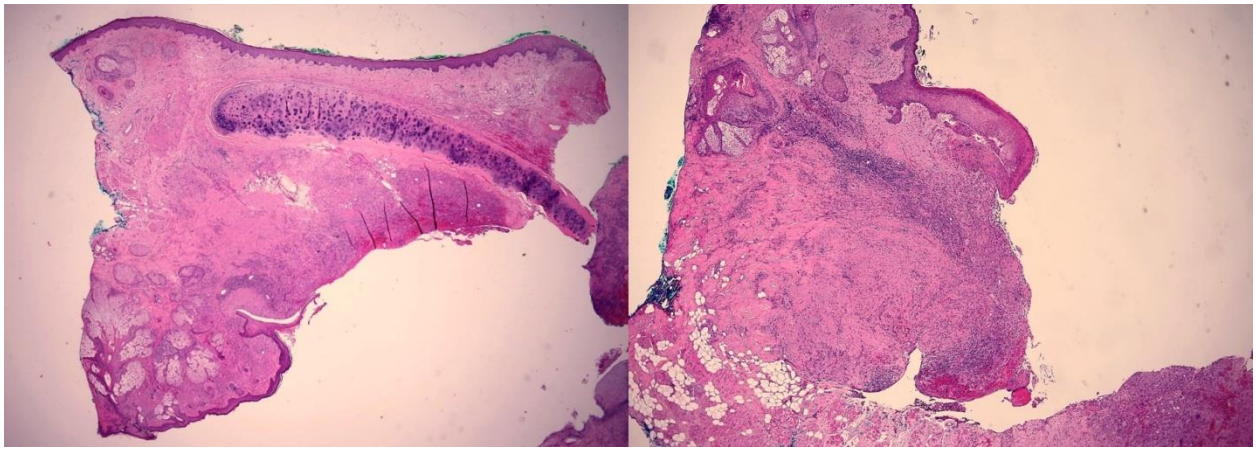
(fig2. Histopathology aspect of lesion as an ulcerated basocellular carcinoma involving the cartilage)



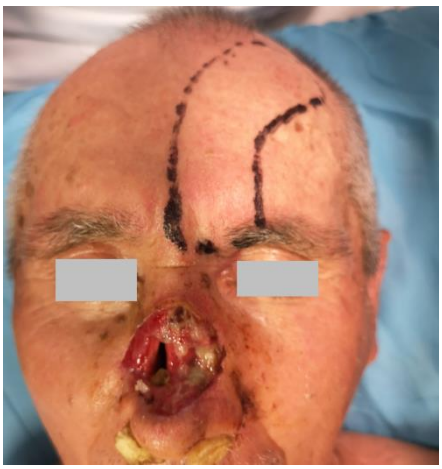
(fig 3 Post tumor excision tulle gras dressing)



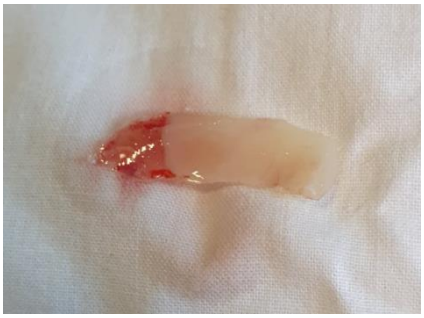
(fig 4. Hp aspect of first re-excision- tumor free)



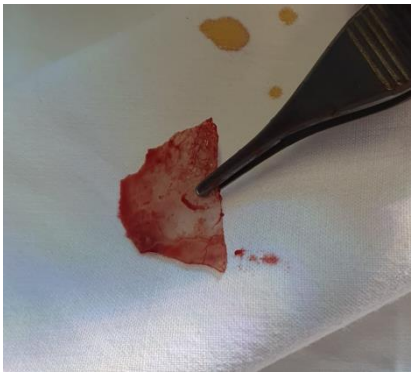
(fig. 5. Second re-excision. Tumor free margins and specimen tissue. Radical treatment was achieved.)



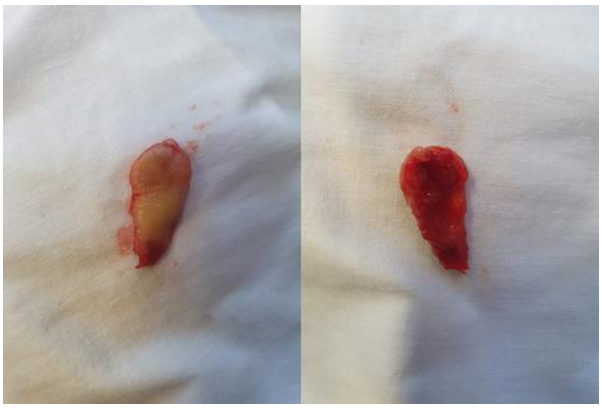
(fig 6. Frontal flap design)



(fig 7. Conchal graft to recreate right alar cartilage)



(fig 8. Inferior septal cartilage graft and bone graft from ethmoidal plate for reconstruction of the anterior septum)



(fig 9. oral mucosal graft)

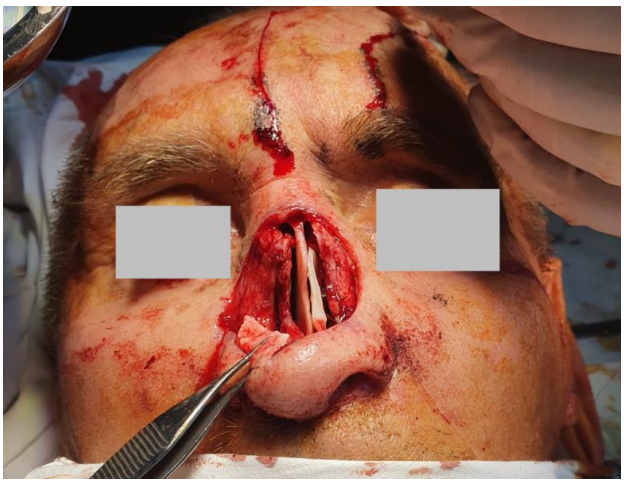
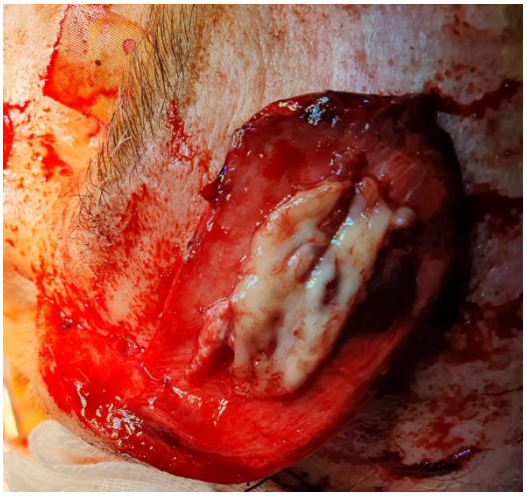
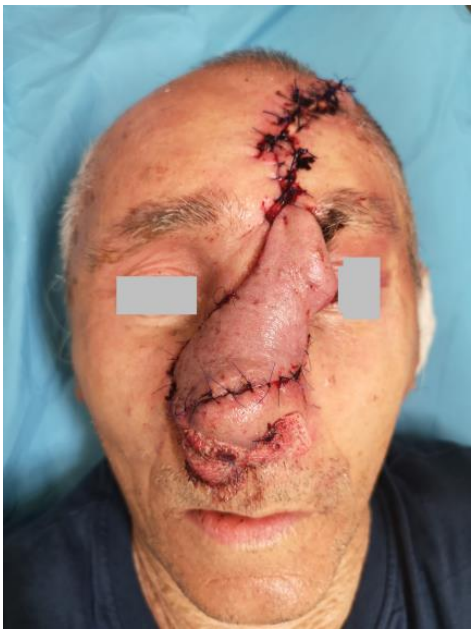


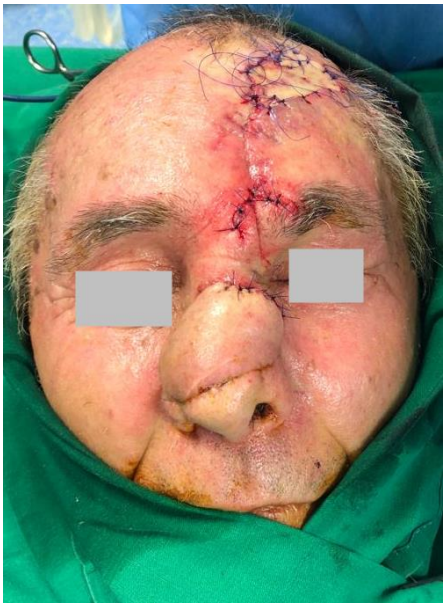
fig. 10- allar cartilages and septum grafts)



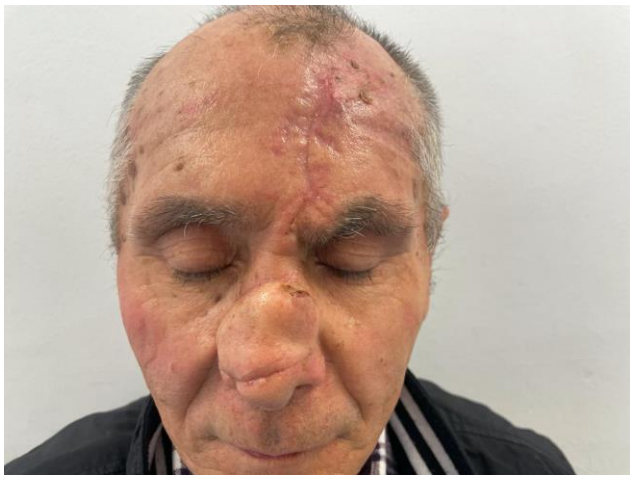
(fig. 11- oral mucosal graft inside the flap)



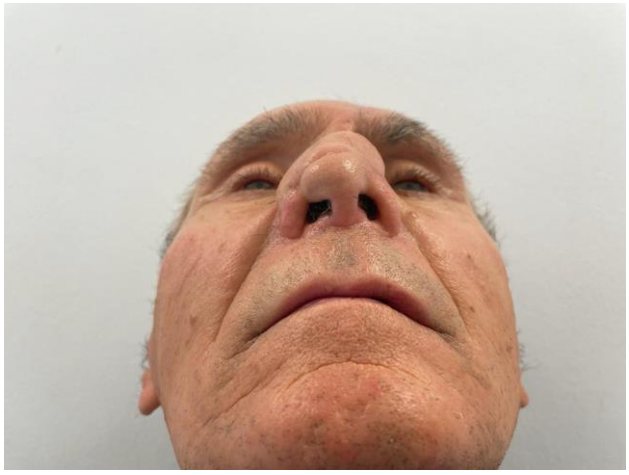
(fig. 12- frontal flap in place after 1st step reconstruction)



(fig 13. Pedicle division and forehead skin graft



(fig 14. Post-op aspect at 2 months. Frontal view



(fig15. Post-op aspect. Nostril shape close to normal, not deviated, not obstructed)

IDENTIFICATION OF *CIS* AND *TRANS* CONFIGURATIONS IN BIOACTIVE LIPIDS USING MASS-ENERGY PROFILES

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ABSTRACT

Lipids are important components of biomass and their molecular geometry has an essential role in ensuring their biological activity. Knowing their correct structure is imperative for providing the proper diagnosis in medicine, or for designing efficient drugs with minimal side effects. The *cis* and *trans* configurations of the C=C bond, responsible for the molecular geometry of lipids, are difficult to establish due to the large number of diastereomers and their possible interconversion. This preliminary study was performed in order to establish whether the quantum calculated mass – fragmentation energy profiles and mass spectra of lipids can be useful in discriminating these configurations. The experimental validation, carried out with oleic and elaidic acids, shows that this type of analysis, based on the coupling of mass spectrometry with quantum-chemical methods, could decode the structural information regarding the *cis-trans* configuration of the C=C bond in lipids.

Keywords: diastereomers recognition, mass spectrometry, mass-energy profiles, quantum chemical calculations

INTRODUCTION

The crucial role of lipids in cell, tissue, and organ physiology is demonstrated by a large number of genetic studies and by the many human diseases that involve the disruption of lipid metabolic enzymes and pathways. Examples of such diseases include cancer, diabetes, as well as neurodegenerative and infectious diseases [1]. Lipids are the main components of the cell membrane, with a decisive role in cell activity through the selective permeability exhibited towards the chemical information [2]. Fatty acids are ubiquitous in the structure of sphingolipids from ceramides, cerebrosides and gangliosides, which are bioactive compounds with a complex role in the body and frequently used in diagnosis [3].

The *cis* configuration of the C=C bond predominates in fatty acids from natural lipids. This aspect is crucial in biochemistry, as it is known and proven that the stereochemistry of an isomer is essential in ensuring its biological activity [4]. This is why the content of *trans* fatty acids in raw alimentary materials and foodstuffs has long been the focus of the European Food Safety Authority (EFSA) because numerous studies indicated them as risk factors for numerous diseases. Thus, the risk of death from heart disease, the highest in the European Union, increases by 20-32% if more than 2% of the total daily diet is provided by *trans* fats. *Trans* lipids accumulate in biomass due to food processing. Chromatography using chemical standards and IR spectroscopy are the only accepted methods for their analysis [5,6]. Due to the fact that a single composition could correspond to lots of isomers, of which only some are biologically active, the difficulty of establishing the true structure inevitably arises even when using these analytical methods. For example, a recent atlas includes for the lipidome no less than 117 lipid subclasses and 8,051 lipids [7]. The linolenic acid group (18:3) alone contains 2912 possible positional and geometrical isomers of the C=C bond. For most of these lipid isomers no standards are available, and either they cannot be identified or a false positive structural assignment is forced upon them. The correct structure is the only one that can be useful in medical diagnosis or for designing efficient drugs with minimal side effects.

In this context, knowing the stereochemistry due to the C=C bond of lipids is essential because the *cis* configuration in lipids generates bends in the hydrocarbon chains, while the *trans* configuration does not change their linearity [6].

The current paper describes a preliminary study aimed at establishing whether recent strategies based on quantitative structure–fragmentation relationship (QSFR) [8, 9] could be useful for this purpose. In these techniques, the mass – fragmentation energy profiles, quantum-chemically calculated for the candidate structures, are compared with the corresponding mass-ion intensity profile from the experimental spectrum of the analyte. The true structure is considered to be

the one that gives the highest match score.

MATERIAL AND METHODS

The applied QSFR method used the mass-energy profiles calculated for two candidate *cis-trans* diastereomers, namely oleic acid (18:9Z) and elaidic acid (18:9E) (Figs. 1 and 2). Method validation was done with positive ion mass spectra recorded over time for standard oleic acids, available in spectral databases.

Mass-energy profiles

Oleic and elaidic acids have the same fragmentation pathways (Figures 1 and 2). The mass series chosen for the profiles correspond to primary positive ions resulting from the ionization and cleavage of a single bond in the candidate structure: S_5, S_7, S_8, S_{10}, S_{11}, OH cleavage, H_2O loss and the molecular ion (Figures 1 and 2).

The enthalpy of molecule fragmentation, corresponding to each of these ions, was calculated by using the RM1 semi-empirical method and the equation:

$$\Delta_f H_{frag} = \Delta_f H(I_i^+) + \Sigma\Delta_f H(F_i) - \Delta_f H(M) \qquad (1)$$

where $\Sigma\Delta_f H(F_i)$ is the sum of the formation enthalpies of the accompanying fragments, $\Delta_f H(I_i^+)$ is the formation enthalpy of the resulting ion and $\Delta_f H(M)$ is the molecular enthalpy of the candidate structure. Both calculated profiles consist of 13 pairs of corresponding ion mass - enthalpy of fragmentation values. The experimental profiles are formed by ion mass – ion intensity pairs.

Quantum calculations

The RM1 semi-empirical method was chosen because it has provided good results in previous studies and is more affordable [9]. All structures were initially modeled using the HyperChem 8.0.10 software. Neutral molecules, obtained after MM+ pre-optimization, were optimized using the RM1 method [10]. The radical cations were obtained from these structures and were finally optimized using RM1. As for "spin pairing", RHF operators were used for neutral molecules and cations while UHF operators were employed for radicals and radical cations. For geometry optimization and $\Delta_f H$ calculation, the "Polak–Ribière (conjugate gradient)" algorithm was selected with an RMS gradient of 0.01 kcal/(Å·mol). The molecules were considered to be placed in vacuum.

Matching profiles

Energy profiles were aligned by using the order of increasing ionic masses (Table 1: B, C and D columns). The match score (P) of the candidate structures' profiles with the experimental profile was calculated with the MS Excel software by using equation (2) in which the linear correlation coefficient R appears.

$$P\ (\%) = 100(1-R)/2 \qquad (2)$$

Their match is one of complementarity [9] since the ionic intensity is inversely proportional to the fragmentation enthalpy, a fundamental principle in chemical kinetics. Relation (2) is advantageous (Table 1, field *f(x)*) because, when the intensities of some ions in the spectrum are missing, the linear correlation function processes only the existing values.

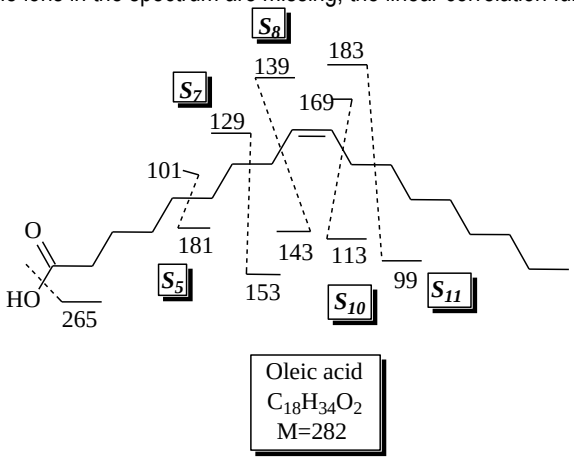

Fig. 1. Ion formation pathways used for the mass-energy profile of oleic acid, the *cis* isomer.

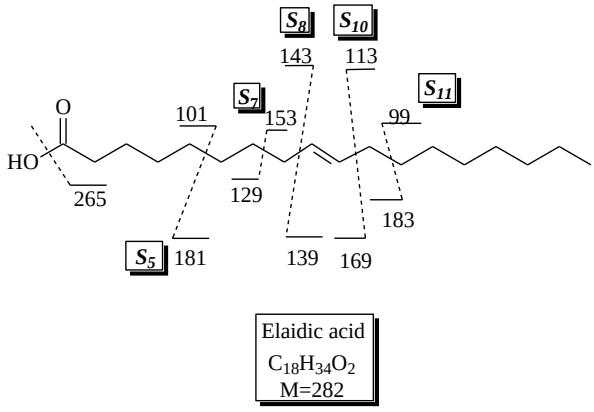

Fig. 2. Ion formation pathways used for the mass-energy profile of oleic acid, the *trans* isomer (elaidic acid).

RESULTS

Table 1 shows the energy values calculated with RM1 and equation (1) (columns B and C), the masses of the ions considered for profiling (column D), the intensities of the respective ions from standard spectra (columns E-M) and the resulting matching scores for oleic acid (row 17) and elaidic acid (row 18).

DISCUSSION

The fragmentations S_7 and S_{11} are allylic (Figures 1 and 2), and the resulting allylic ions (*m/z* 99, 129, 153, 183) are stabilized by conjugation. Quantum calculations show that the enthalpies of allylic fragmentation for oleic acid are higher than those for elaidic acid (Tab. 1), in agreement with the stabilization of allylic ions, greater in the *trans* than in the *cis* configuration. In the case of the molecular ion (*m/z* 282) the situation is also similar. The higher scores obtained for oleic acid (formatted green in Table 1) for eight out of the nine standards, indicate the true structure, thus validating this method of discrimination for *cis-trans* diastereomers based on mass-energy profiles calculated with RM1. Its further optimization can improve its accuracy and selectivity.

E17 =100*(1-CORREL(E4:E16,$C4:$C16))/2

	B	C	D	E	F	G	H	I	J	K	L	M
2	$\Delta_f H_{frag}$ (kcal/mol)			Ionic currents (arbitrary units)								
3	Elaidic acid	Oleic acid	MS ions	#133071: 9-Octadecenoic acid (Z)- (CAS)	#133072: 9-Octadecenoic acid (Z)- (CAS)	#133073: 9-Octadecenoic acid (Z)- (CAS)	#133074: 9-Octadecenoic acid (Z)- (CAS)	#133075: 9-Octadecenoic acid (Z)- (CAS)	#133076: 9-Octadecenoic acid (Z)- (CAS)	#133077: 9-Octadecenoic acid (Z)- (CAS)	#133078: 9-Octadecenoic acid (Z)- (CAS)	#133080: 9-Octadecenoic acid (Z)- (CAS)
4	244.8	245.4	**99**	800	590	268						340
5	259	258.8	**101**	900	870	357						430
6	270.4	270.5	**113**	500	410							210
7	246.6	247.2	**129**	2900	1190				1140			370
8	269.6	268.3	**139**	300	400	268	490					230
9	271.7	271.9	**143**	700	210							100
10	232.2	234.3	**153**	200	200		240					140
11	270.4	270.5	**169**		70							60
12	254.3	254.3	**181**	100	50							50
13	233	235.2	**183**		20							30
14	229.3	229.2	**264**		410	1339	1120	300	420	864	1900	300
15	264.7	264.7	**265**		100	268	220	100	82	169	372	100
16	199.8	201.5	**282**		50	268	160	40	60	62	500	50
17	**SCORE (%) Oleic acid →**			**59.5**	**44.4**	**63.9**	**49.4**	**42.5**	**36.8**	**47.4**	**57.3**	**45.4**
18	**SCORE (%) Elaidic acid →**			59.3	43.9	63.2	48.2	41.6	**36.8**	46.5	56.4	44.7

Table 1. MS Excel page for calculating the matching score (rows 17 and 18) between the calculated mass-energy profiles for oleic and elaidic acids (columns B and C), and the mass-ion intensity profiles from the reference mass spectra (columns E-M)

CONCLUSION

The relativity of the primary ions' intensities in the mass spectra of fatty acids contains structural information regarding the *cis-trans* configuration of the C=C bond. Mass – fragmentation energy profiles, calculated with the quantum method RM1, can serve to decode this structural information. This QSFR method can be further developed for its use in the study of the chemical structure-biological activity relationship, in which lipid chain geometry is involved.

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METODĂ DE IDENTIFICARE A CONFIGURAŢIILOR *CIS* ŞI *TRANS* ÎN LIPIDELE BIOACTIVE UTILIZÂND PROFILURI MASĂ-ENERGIE

REZUMAT

Lipidele sunt componente importante ale biomasei iar geometria lor moleculară are un rol esențial în asigurarea activității biologice. Cunoașterea structurii lor corecte este singura care poate fi utilă în diagnostic sau proiectarea de medicamente eficiente cu efecte secundare minime. Implicate în geometria moleculară a lipidelor, configurațiile *cis* și *trans* ale legăturii C=C se stabilesc anevoios din cauza numărului mare de diastereomeri și interconversiei posibile a acestora. Lucrarea noastră face un studiu preliminar pentru a stabili dacă profilurile masă-energie de fragmentare calculate cuantic și spectrele de masă ale lipidelor pot fi utile la discriminarea acestor configurații. Validarea experimentală, realizată cu acizii oleic și elaidic, arată că acest tip de analiză, bazat pe cuplarea spectrometriei de masă cu metodele cuanto-chimice, ar putea decodifica informația structurală referitoare la configurația *cis-trans* a legăturii C=C din lipide.