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REVIEW

ROLE OF CEREBRAL IMAGING IN THE MINIMALLY INVASIVE TECHNIQUES FOR INTRACEREBRAL HEMATOMA EVACUATION

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Abstract. ***Introduction.*** *Intracerebral hemorrhages represent the second most frequent, but the most severe form of stroke, with 1 in 3 patients passing away shortly after its debut. Considering these data, it is necessary to identify efficient ways to evacuate intracerebral hematomas and improve their morbidity and mortality, with brain imaging being truly helpful to neurosurgeons.* ***Objective****s. To identify the role of imaging for the evacuation of spontaneous intracerebral hematomas.* ***Methods****. We performed an extensive literature review, examining the latest published studies and therapeutic protocols. We performed a comprehensive evaluation of the latest imaging and surgical techniques for the diagnosis and treatment of intracerebral hemorrhages.* ***Results****. These studies suggest that surgical intervention and evacuation of the hematoma, based on imaging and clinic, can have an immediate life-saving effect on certain groups of patients, but it does not significantly influence the long-term prognosis and death rate.* ***Conclusions****. Modern imaging techniques help neurosurgeons preoperatively, as they can more accurately estimate the benefits of the surgical intervention, intraoperatively through neuronavigation, and postoperatively, modulating therapeutic management by identifying specific imagistic signs. Surgical interventions, both invasive and especially minimally invasive, have a proven positive effect on the evolution of patients, reducing acute mortality, but with uncertain results regarding improving long-term prognosis.*

Keywords: *spontaneous, intracerebral hemorrhage, stroke, hematoma, neurosurgery, radiology.*

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Abbreviations

ICH, intracerebral hemorrhage; CT, computed tomography; MRI, magnetic resonance imaging; AHA/ASA, American Heart Association/ American Stroke Association; CTA, CT angiography; HU, Hounsfield units; AVM, arteriovenous malformation; DSA, digital subtraction angiography; SAH, subarachnoid hemorrhage; TNF, tumor necrosis factor;

INTRODUCTION

Strokes are an important cause of global mortality, responsible for approximately 5.5 million deaths annually [1]. Intracerebral hemorrhages (ICH) represent 10-20% of all cerebrovascular events but account for almost 40% of all deaths, with first-month mortality reaching 40% [2-4]. Despite the technological-related evolution of the treatment of this pathology, there is no conclusive data to prove the increase in survival after an episode of cerebral bleeding [5]. A meta-analysis showed a survival rate of 46% at 1 year and 29% at 5 years [6].

There are primary and secondary cerebral hemorrhages [4]. Atherosclerotic angiopathy and arterial hypertension are the primary causes of hemorrhage, whereas vascular structural abnormalities or anticoagulant therapies are the secondary causes.

ACUTE IMAGING DIAGNOSIS OF ICH

A quick imaging examination is essential for the diagnosis and management of patients with intracerebral hemorrhage. Rapid examination by computed tomography/ magnetic resonance imaging (CT/MRI) is the first-line recommendation by the American Heart Association and American Stroke Association (AHA/ASA) guidelines [7].

The evolution of hematoma is divided into 5 stages: hyperacute (<12 hours), acute (12-48 hours), early subacute (2-7 days), late subacute (8-30 days), and chronic (>30 days) [8].

Usually, in the hospital, the first-choice imaging examination is a non-enhanced brain CT. This method is affordable, simple, quick, widespread, and has excellent sensitivity for ICH, being able to identify and measure the volume of the hematoma, the presence of cerebral edema and the midline shift (Figure 1), intraventricular extension or the presence of hydrocephalus (Figure 2, Image C) [9]. It is considered the gold standard for the diagnosis of ICH in the emergency room [10]. There are several specific signs by which the hematoma expansion can be detected using non-contrast CT: hypodensities in the area of the hematoma, a swirl sign, a black hole sign, a blend sign, an irregular shape, and heterogeneous densities [11]. These signs have low sensitivity (below 50%) but high specificity (up to 95% in the case of the black hole sign) [11].

Some distinct imagistic patterns can facilitate the differential diagnosis of the underlying pathology that led to ICH. Thus, multiple hemorrhages of different ages suggest amyloid angiopathy; different-aged hemorrhages within the same hematoma suggest hemorrhage induced by anticoagulants; the combination of small ischemic and hemorrhagic lesions suggests vasculitis [8].

According to the AHA/ASA guidelines, contrast or non-contrast brain CT or CT angiography are the next investigations to follow to identify hematoma expansion [7]. These techniques can also help in the identification of the primary cause of an ICH. The spot sign is one of the most reliable signs that prove hematoma expansion and, subsequently, active extravasation of blood, represented by small enhancing areas of about 1-2 mm superimposed in the area of the hematoma, adjacent to vessels, indicating which patients could benefit from (micro)surgical intervention [12-14]. The incidence of this sign is 17-56% [13]. A recent meta-analysis suggests that its presence may reflect a poor prognosis and increased mortality [15].

I. Computed tomography (CT)

According to the AHA/ASA guidelines, CT is the gold standard, quick, cheap, and feasible ICH evaluation method for unstable patients [7]. The hematoma density varies depending on the clinical stage of the hemorrhage. Thus, in the hyperacute phase, the densities vary in the range of 30-60 Hounsfield units (HU), while in the acute phase, they tend to increase to values of 100 HU [16]. In the case of abnormal hematocrit values, HU may vary [17].

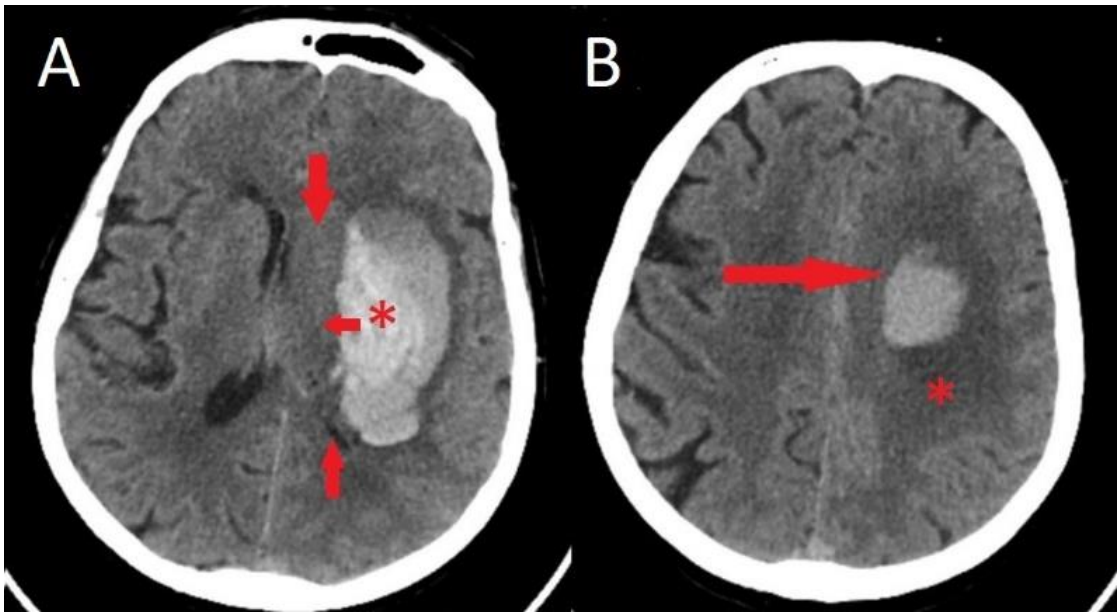

Figure 1. *Non–enhanced CT scan of a patient with an acute ICH. A spontaneously hyperdense, heterogeneous lesion with hematic densities, at the level of the left insular cortex and internal capsule (Image A, *) compressing the basal nuclei and lateral ventricle (Image A, arrows), extended subcortically in the frontotemporal region, the left corona radiata and ipsilateral semioval centrum (Image B, arrow), with a mass effect, shifting the midline with about 9 mm. Perilesional edema with a thickness of up to 10 mm surrounds the hematoma (Image B, *)*

In the subacute phase, the lesion will become parenchymatous-isodense, requiring brain MRI for clear differentiation [18]. The hematoma volume can be estimated by using the ABC/2 formula, where A represents the longest axis, B is perpendicular to this axis, and C is the number of slices in which the hematoma is found multiplied by their thickness or the height of the hematoma [19]. Over 60 ml of volume is associated with high mortality [20]. About one-third of the ICH CT-diagnosed patients will show hematoma expansion in the first 3 hours after the symptomatology onset [7].

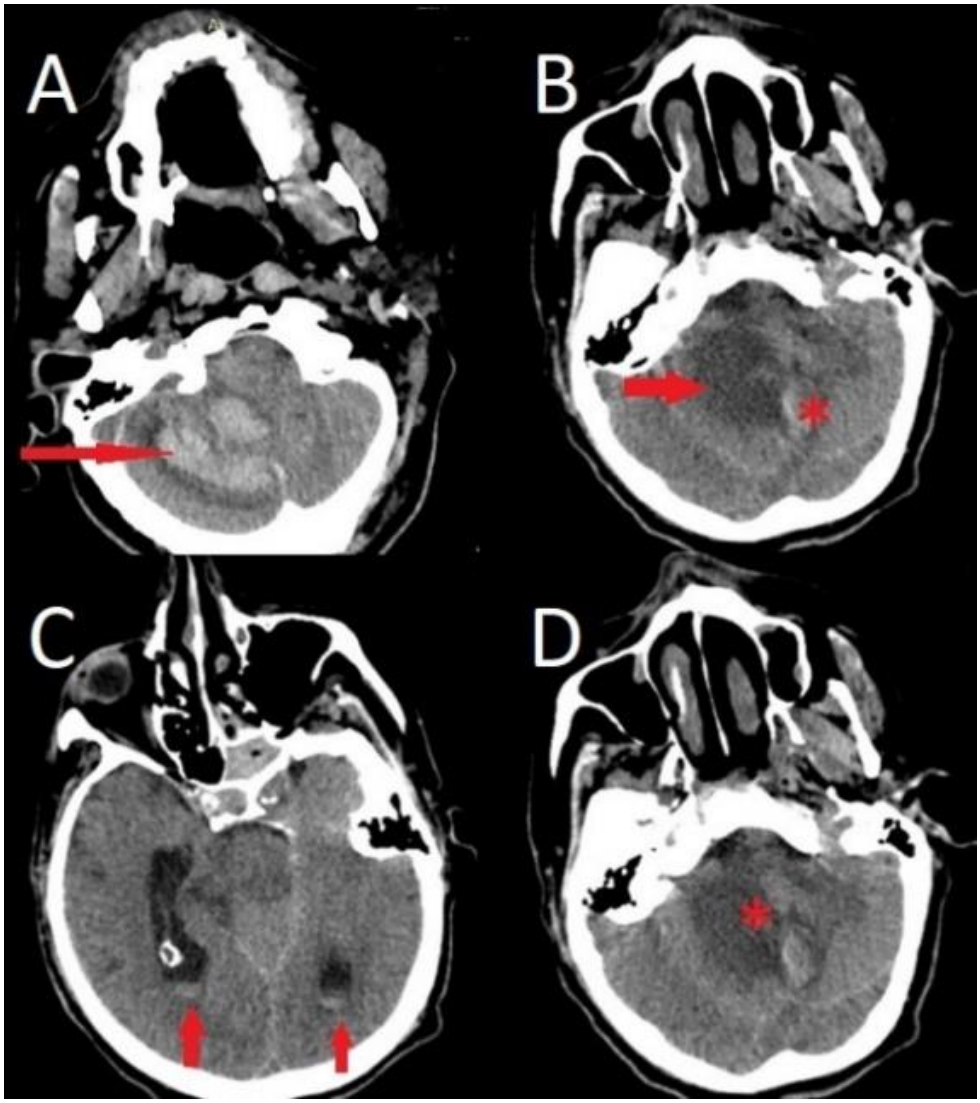

***Figure 2**. Infratentorial hemorrhage with multi-level expansion. Spontaneous hyperdense accumulation, with blood-like densities, in the anterior 2/3 of the right cerebellar hemisphere (image A, arrow) with periventricular extension and at the level of the left cerebellar hemisphere (image B, *), associating neighbouring parenchyma edema (image B, arrow), breaching the lateral ventricles (image C, arrow) and with significant mass effect on the normal parenchyma (midline shift up to 13-14 mm), the 4th ventricle and the medulla oblongata with basal cisterns obstruction (image D, *)*

Several signs that can predict the evolution of a patient with ICH have been described. The black hole sign (Figure 3, B) was defined as a hypoattenuating area encapsulated in the hyperdense hemorrhagic area. Similarly, the blend sign is described as the blending of a well-defined hypodense area within the hematoma area. The latter can be useful on the non-contrast CT examination when CTA is unavailable, demonstrating a high predictive value of neurological damage, similar to that of the spot sign.

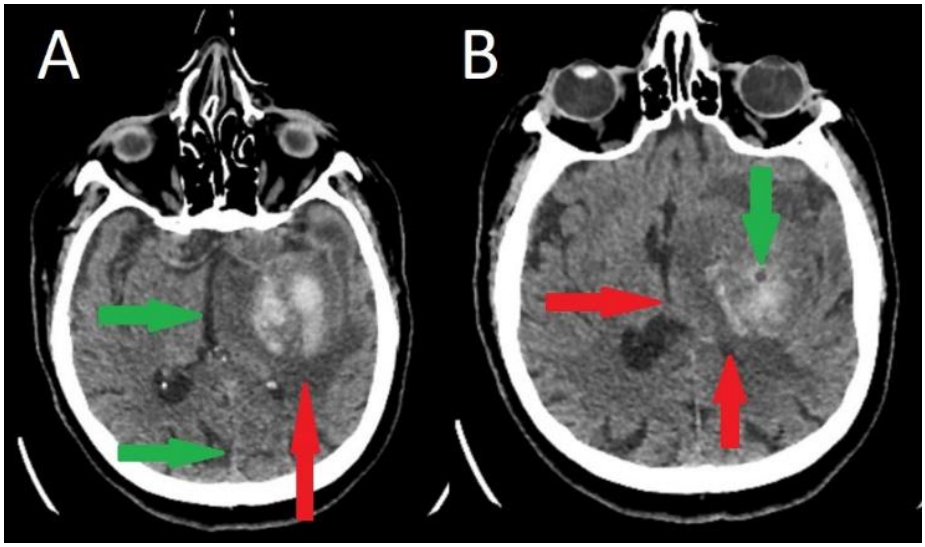

***Figure 3**. Heterogeneous intraparenchymal hematoma located in the left hemisphere, with significant perilesional edema (Images A and B, red arrow), midline shift (Image A, green arrows), involvement of the ipsilateral thalamus and compression of the lateral and third ventricle (Image B, red arrows). Black hole signature (Image B, green arrow).*

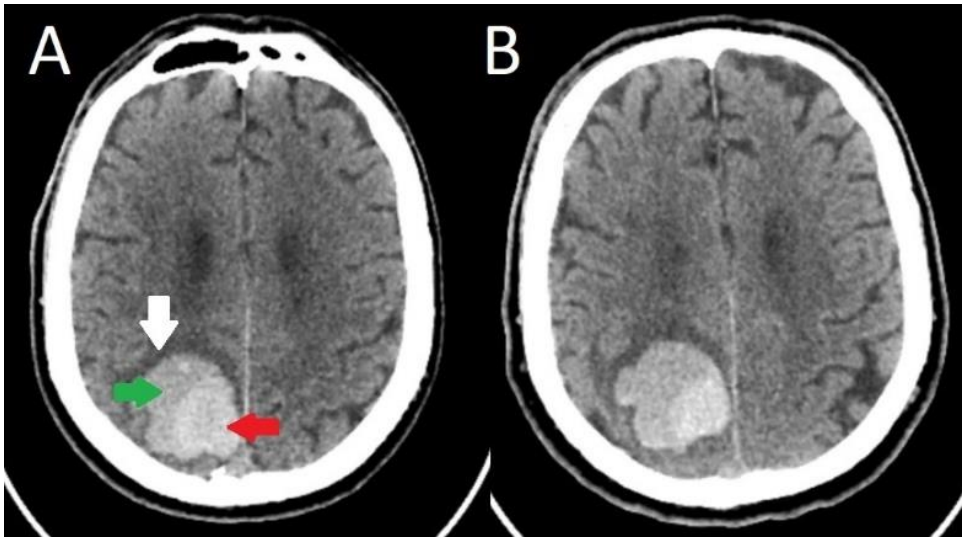

***Figure 4**. Right parietal-temporal intraparenchymal hematoma in the acute-subacute stage with heterogeneous appearance, suggesting blood products in different stages of evolution (blend sign - Image A, green and red arrows), associating perilesional edema (Image A, white arrow) but without deviation of the median line. Image B shows a stationary aspect 4 days after the onset of symptoms.*

Among these techniques' disadvantages are the difficulty of highlighting hemorrhage in a patient with < 10g/dl of Hgb, the posterior fossa evaluation being difficult due to artifacts, or the omission of thin blood collections due to partial volume artifacts.

The most common sites of intraparenchymal hemorrhage are capsular (40%) (Figure 5), lobar (30%), thalamic (10%), cerebellar (10%), pontine (7%), and intraventricular (3%) (Figure 5) [12].

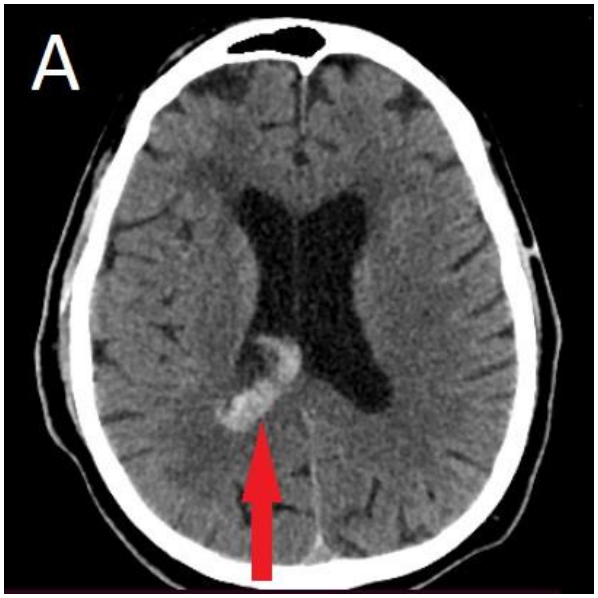

Figure 5. *Intraventricular hemorrhage. Spontaneous hyperdense image at the level of the body and atrium of the right lateral ventricle with a thickness of up to 6 mm, without obstructive hydrocephalus.*

Depending on the results and the bleeding patterns, further imaging tests may be necessary: repeating the CT scan to identify hematoma expansion, midline shift, or cerebral edema; CT angiography (CTA) or digital subtraction angiography (DSA) for subarachnoid hemorrhage (SAH) or arterio-venous malformation (AVM) related to hemorrhage. Unenhanced brain MRI for suspected hemorrhagic transformation (HT) of an ischemic stroke; contrast-enhanced brain MRI when suspicioning an underlying tumor or an infectious process; CT venogram in case of suspected venous thrombosis [21].

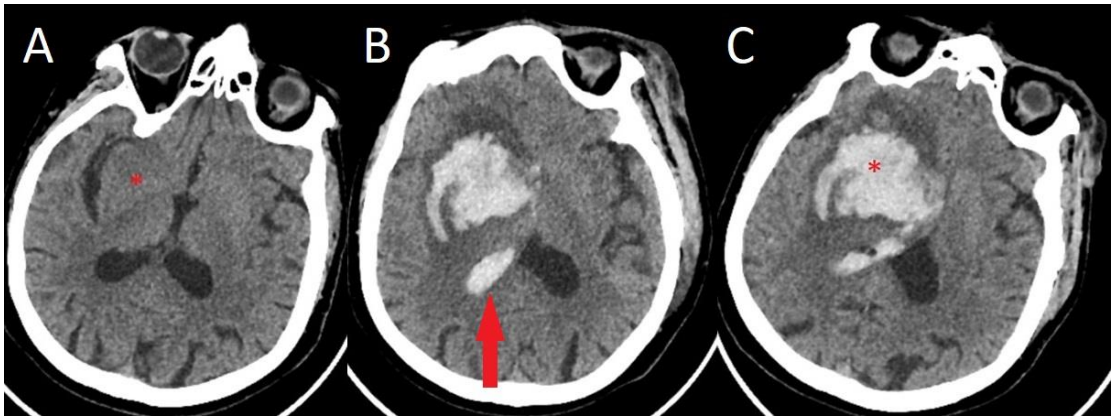

Figure 6. *Reassessment 24 and 48 hours after the onset of an ischemic stroke. Image A. Ischemic stroke at the level of the basal ganglia and internal capsule on the right side (*); Image B. The worsening of the symptoms led to a second CT examination, 24 hours after the onset of the symptoms, which revealed the significant dimensional hemorrhagic transformation associated with a midline shift contralaterally by 12 mm and ventricular effusion at the level of the occipital horn of the left ventricle (arrow). Image C. The evolution of the hematoma 48 hours after the onset of ischemic symptoms shows minimal dimensional evolution of the hematoma.*

II. Magnetic resonance imaging (MRI)

Even though the CT exam is considered the gold standard, the HEME study demonstrated that MRI can even exceed the sensitivity of CT in certain cases, such as highlighting chronic bleeding or microhemorrhages through magnetic susceptibility sequences [22,23]. Also, MRI is essential to perform a differential diagnosis between the two most common causes of intracerebral hemorrhage: amyloid angiopathy and hypertensive vasculopathy [24]

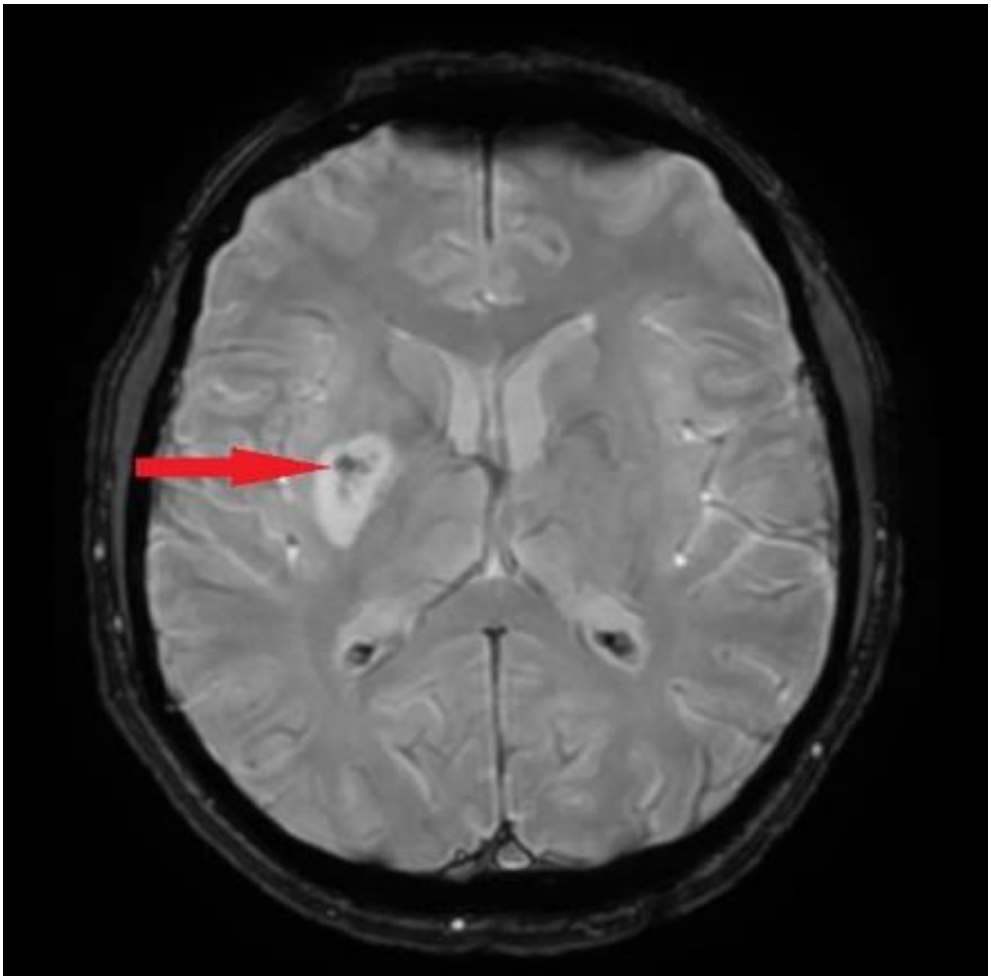

Figure 7*. Evidence of magnetic susceptibility changes on the SWI sequence (arrow) with a suggestive appearance for the hemorrhagic transformation of a deep ischemic stroke, in the capsule-lenticular region.*

In the hyperacute phase, the hematoma is isointense on the T1-weighted sequence, and hyperintense on the T2-weighted and FLAIR sequences. In the acute phase, the lesion is isointense on the T1-weighted sequence and markedly hypointense on the T2-weighted and FLAIR sequences. In the subacute phase, the lesion shows hyperintensities on T1-weighted, T2-weighted, and FLAIR sequences, and in the chronic phase, the T2-weighted hyperintense center is surrounded by a hypointense ring, the images being in the mirror with the FLAIR sequence (hypointense centre, hyperintense ring) [25]. On magnetic susceptibility sequences, in all phases, the lesion is hypointense or markedly hypointense.

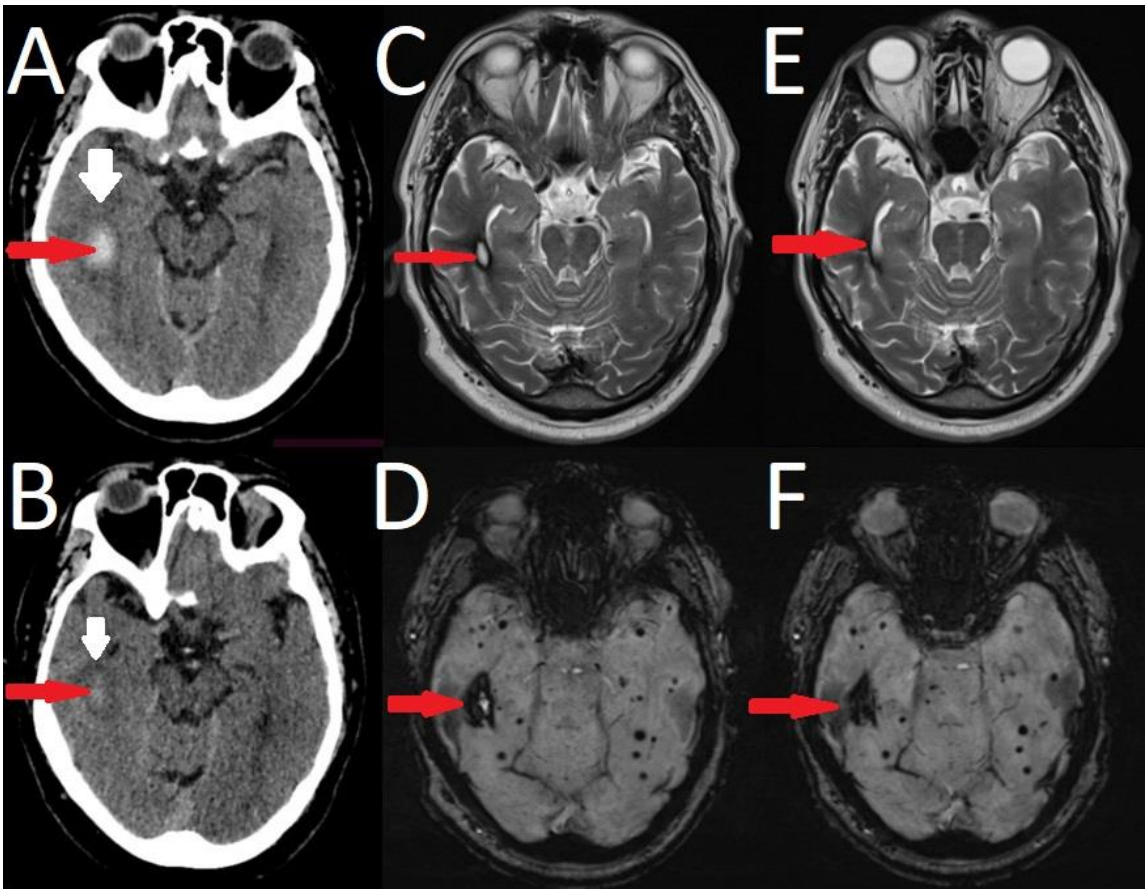

Figure 8*. Image A. Acute right temporal ICH (red arrow) with peripheral edema (white arrow); Image B. Partial resolution of the hematoma and peripheral edema at 7 days; Images C and D. MRI exam 2 months after the onset of symptoms. The T2-weighted sequence (image C, red arrow) shows a partially resolved hematoma while the susceptibility-weighted sequence (image D, red arrow) shows a globulous deposit of hemosiderin. Images E and F. Another MRI exam 3 months after the onset of symptoms. T2-weighted sequence (image E, red arrow) shows complete resorption of the hematoma, while the SWI sequence (image F, red arrow) has minimal changes. Besides the right temporal hematoma, the patient had lots of SWI-hypointense lesions with lobar and non-lobar distribution (Images D and F)*

III. CT angiography (CTA)

CT angiography is the most frequently used imaging method for cerebrovascular evaluation, having a high sensitivity and specificity for the detection of vascular anomalies [7,26]. Besides these, CTA can select patients at risk by identifying extravasation of the contrast in the volume of the hematoma, known as the spot sign [27].

On the other hand, the use of CTA is limited by its elements: radiation and contrast. The contrast agent can lead to acute renal injury in patients with renal comorbidities, while radiation limits the number of possible exposures.

Two clinical scores, the Intracerebral Hemorrhage (ICH) score and the Bleeding assesment Practical (BAT) score (Tables 1 and 2), are used to more accurately predict the possibility of hematoma expansion, with two others being optional, namely BRAIN and a score described by Browers *et al.* [28,29,32].

Table 1. Bleeding assesment Practical (BAT) score.

Variable	Points
Blend sign	
Present	1
Absent	0
Hipodensities	
Present	2
Absent	0
Time from onset to CT scan	
<2,5h	2
>2,5h	0

A BAT score >3 is correlated with hematoma expansion

Variable	Points
GCS score	
3-4	2
5-12	1
13-15	0
ICH volume, cubic cm	
>30	1
<30	0
IVH	
Yes	1
No	0
Infratentorial origin of ICH	
Yes	1
No	0
Age	
>80	1
<80	0

An ICH score of 0 has a minimum risk of mortality. A score of 6 means an over 95% risk of mortality.

Table 2. Intracerebral Hemorrhage (ICH) score.

IV. Neuronavigation

Neuronavigation is an indispensable system in neurosurgery [33]. The technological advances of the last decades are expressed most strongly in the field of imaging, and actions unthinkable a few decades ago can usually be achieved today thanks to these implemented technological innovations. The improvement of the quality of preoperative imaging, combined with the integration of imaging techniques in mini-devices, has allowed, in the last 30 years, the migration of some imagistic equipment into the operating room and its use even during surgical interventions, thus becoming an irreplaceable aid for neurosurgeons. The evacuation of intraparenchymal hematomas is one of the branches of neurosurgery that has benefited the most from these innovations, allowing a minimally invasive approach. Current neuronavigation systems allow a precision of a few millimeters [34,35]. Using classic neuronavigation systems, imaging scans could be displayed in real-time, 2D, on screens directly in the operating room. In recent years, however, the focus has been on the implementation and use of intuitive 3D augmented reality systems, involving the intuitive superposition of virtual images over the real-world images obtained by cameras. Some of these even allow the projection of relevant data, such as target lesions or operator planning, directly in the operator's visual field, without being distracted by other elements (such as other display systems) [36-38]. These systems identify and adapt the projected data, in real-time and depending on the operator's progress.

V. TREATMENT

The results of conservative treatment of a cerebral hemorrhage are well defined [39]. ICH care can be improved by treating the patient in a neurological intensive care unit, but a better prognosis has not been demonstrated [40].

Two therapeutic approaches are taken into account: the conservative one and the surgical one. We will approach the surgical one.

The role of early surgery in ICH is controversial. There have been several studies conducted over the years to quantify the effectiveness of this therapeutic method. The best known are the STICH study, from 2005, and its continuation, STICH II, from 2013. These two studies have not demonstrated the expected surgical benefits in the case of intracerebral hemorrhages [41,42].

There are lots of minimally invasive surgical techniques that can be applied to patients with intracerebral hemorrhages, but regardless of whether we are talking about minimally invasive surgery, thrombolytic techniques, or non-thrombolytic techniques, the results are still equivocal when we refer to prognosis improvement [43]. The MISTIE study, designed to examine the applicability of minimally invasive surgery and its impact on the reduction of side effects after craniotomy, is one of the most important of its kind. The MISTIE III study, published in 2019, included 499 patients with intracerebral hemorrhages with a volume greater than 30 ml, treated minimally invasively in 78 centers around the world [44]. A good prognosis was obtained in patients with a reduction of the hematoma volume by >53% or a decrease in its volume below 30 ml.

The most common minimally invasive techniques for cerebral hematomas are stereotactic aspiration with thrombolysis [44,45], endoscopic evacuation of cerebral hematomas [46], craniopuncture (the most used technique in China) [47,48], evacuation through endoport [49, 50] or evacuation through the surgiscope [51].

DISCUSSION

Intraparenchymal hemorrhage is, most of the time, a multifactorial result of multiple untreated chronic pathologies; therefore, the discovery of a standard treatment is not feasible, with each patient having to be treated differently.

The poor prognosis is due to the primary injury and, mainly, to the physiopathological responses of the body that materialize through the secondary injury. The inflammatory response from secondary injury is mainly due to interleukins and TNF-α [52].

Arterial hypertension is a comorbidity that is frequently associated with deep hemorrhages, but less so with intralobular hemorrhages, the latter being associated with amyloid angiopathy, according to the Boston criteria [53-55].

Despite the initial hypothesis, which suggests that surgical intervention for the evacuation of intraparenchymal hematomas will decrease the degree of morbidity and mortality, the reality is different. Multiple large studies have obtained equivocal results, with some authors questioning the benefits of the intervention compared to conservative, medical management. It has been demonstrated that surgery in the hyperacute phase is associated with recurrent bleeding, and the prognosis can be affected by several factors such as the surgical technique or the location and size of the hematoma [56]. In the opinion of some authors, endoscopic and minimally invasive interventions have comparable efficiency when their goal is to evacuate the hematoma and decompress the brain, considering that the interventional injury is much lower than the injury caused by hematic degradation [57]. Despite some large cascade studies, the results remained ambiguous, largely due to the heterogeneity of the patient groups [42,58]. However, the STICH II study concludes that the clinical benefits of minimally invasive surgery are minimal but relevant [42]. In another series of studies, comparing minimally invasive surgical interventions with conventional ones,

aspiration with thrombolysis determined the dimensional reduction of the hematoma in up to 70% of cases, with a hematoma size of less than 15 ml correlating with an improvement in the mortality rate at 1 year [44,59].

At this moment, it is difficult to recommend surgical intervention for the evacuation of intraparenchymal hematomas, even with all the data obtained from the multitude of existing studies [57].

CONCLUSIONS

Pre- and intraoperative imaging play a crucial role in the diagnosis and neurosurgical treatment of spontaneous intracerebral hemorrhages. Despite contradictory data in the literature, there is a tendency to recommend minimally invasive interventions for the treatment of hemorrhagic events, with their benefits in improving long-term mortality slightly outweighing the risks of the intervention itself. Next, collaboration within the multidisciplinary team is essential, as the patient should be evaluated and balanced quickly in the emergency and intensive care departments, so that the therapeutic management is then established by a team made up of a neurologist, neurosurgeon, and radiologist, this being, of course, individualized for each patient depending on the clinical condition and the paraclinical data obtained.

Author contributions:

R.I.D conceived the original draft preparation. G.S.T and C.A.S. were responsible for the conception and design of the review. R.I.D. and G.S.T. were responsible for the data acquisition. C.A.S. was responsible for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review. All authors contributed equally to the present work, and have read and agreed with the final version of the manuscript.

Compliance with Ethics Requirements:

The authors declare no conflict of interest regarding this article.

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REVIEW

THE GUT-BRAIN AXIS: THE CORRELATION BETWEEN STRESS AND GUT MICROBIOME

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Abstract. *Although it was thought that the gut microbiome affects gut physiology only locally, it becomes clearer that these trillions of organisms that reside in the gastrointestinal tract of a human being have a more complex function. Preclinical studies have shown that the microbiome has the ability to interact with the brain in various ways. There have been at least three different channels of communication that favour bidirectional interaction between the brain and the gut. The aim of this review is to summarize the connection between the gut microbiome and the brain, highlighting the process in which stress, in its various forms, can affect the homeostasis of the gastrointestinal tract. Modifications in the gut-brain-microbiome interactions have been analysed and determined in several rodent models of digestive and neurological disorders. The manner in which this information can apply to human beings, is yet to be discovered. Taking all things into account, it is clear that a better understanding of this means of communication could open the door for future therapies for gastrointestinal conditions.*

Keywords: *gut microbiome, gut-brain interactions, microbial signalling, stress-induced alterations.*

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Abbreviations: GB= Gut-Brain; GI= Gastro-intestinal; IBS= Irritable bowel syndrome; ENS= Enteric nervous system; CNS= Central nervous system; ANS= Autonomic nervous system; SCFAs= Short-chain fatty acids; 2Bas= Secondary bile acids; TLRs= Toll-like receptors; MAMPs= Microbe-Associated Molecular Patterns; HPA= Hypothalamic pituitary-adrenal; SPF= Specific pathogen-free; GF= Germ free; BMI= Body-mass index; CFU= Colony forming unit; MODS= Multiple organ dysfunction syndrome; SIRS= Systemic inflammatory response syndrome; L/D test= Light-Dark box test.

1. Introduction

Discussions revolving around the importance of the human microbiome have recently gained great popularity among scientists. Although questions regarding the subject continue to emerge, there is yet not sufficient information to define the exact mechanisms and pathophysiology of how the gut microbiome influence general health. As in present days, the mental health of the human population has become extremely valued, as a great number of people struggle with problems in day-to-day life. For that reason, it is imperative to learn more about the correlation between stress and the gut microbiome, as well as the latent issues that

can arise from the imbalance of the microbial species in the human intestines.

2. The importance of gut microbiome

The human gastrointestinal system is home to trillions of bacteria, viruses, fungi, archaea, and eukaryotic species, which are together referred to as the gut microbiome. These organisms, which may weigh up to 2 kg in an ordinary adult, account for more than 50% of the cells in the human body [1]. Due to the acidic environment, the presence of bile and pancreatic juice, and the effects of peristalsis that prevent sustained colonization, the stomach and small intestine contain few organisms. Therefore, the vast majority of the microbiota reside in and communicate with the human host in the colon. In the past ten years, due to increasing availability and lower costs, our understanding of the human gut microbiome in both health and illness has dramatically risen [2].

Even though one cannot entirely define a healthy microbiome, there are a few standards that one may consider. Relevant characteristics are the diversity of the microbial species, stability, resistance to stress-related change (antibiotics, infection, immunosuppression), and a high level of redundancy in metabolic pathways [3].

3. The gut-brain axis

Food intake, immune function, and sleep are all regulated by bidirectional interactions between the gut and the brain (GB). Even though modifications of GB interactions have for quite some time been hypothesized to be the cause of chronic abdominal pain symptoms and gastrointestinal dysfunction, the correct terminology (disorders of the GB interaction instead of functional GI disorders) has only recently been acknowledged by experts [4]. Even though research over the past ten years has accomplished significant information about the pathophysiology of GB disorders like irritable bowel syndrome (IBS) and functional dyspepsia, there is still disagreement about the relative contribution of peripheral (such as the gut) and central (such as the brain, spinal cord) mechanisms to the generation of symptoms in these diseases and other comorbid syndromes like functional chest pain and functional abdominal pain. Nonetheless, there is developing agreement that the pathology of persistent stomach pain can be understood as a dysregulation of the interaction between signalling in the stomach, intestinal microbiota, enteric nervous system (ENS), and central nervous system (CNS). This process is believed to occur at the same time with modifications in gut motility and regional transit, visceral sensitivity, immune function, and mood [5].

The newly discovered interaction between the gut and the brain is thought to be responsible for different brain disorders whose pathophysiology had been formerly correlated to mechanisms limited to the brain. Preclinical and clinical studies suggest that treatment of gut dysbiosis could become a reasonable solution for defective GB interactions, as well as for psychiatric and neurological disorders (depression, anxiety, Alzheimer's disease, Parkinson's disease, and autism spectrum disorders [5].

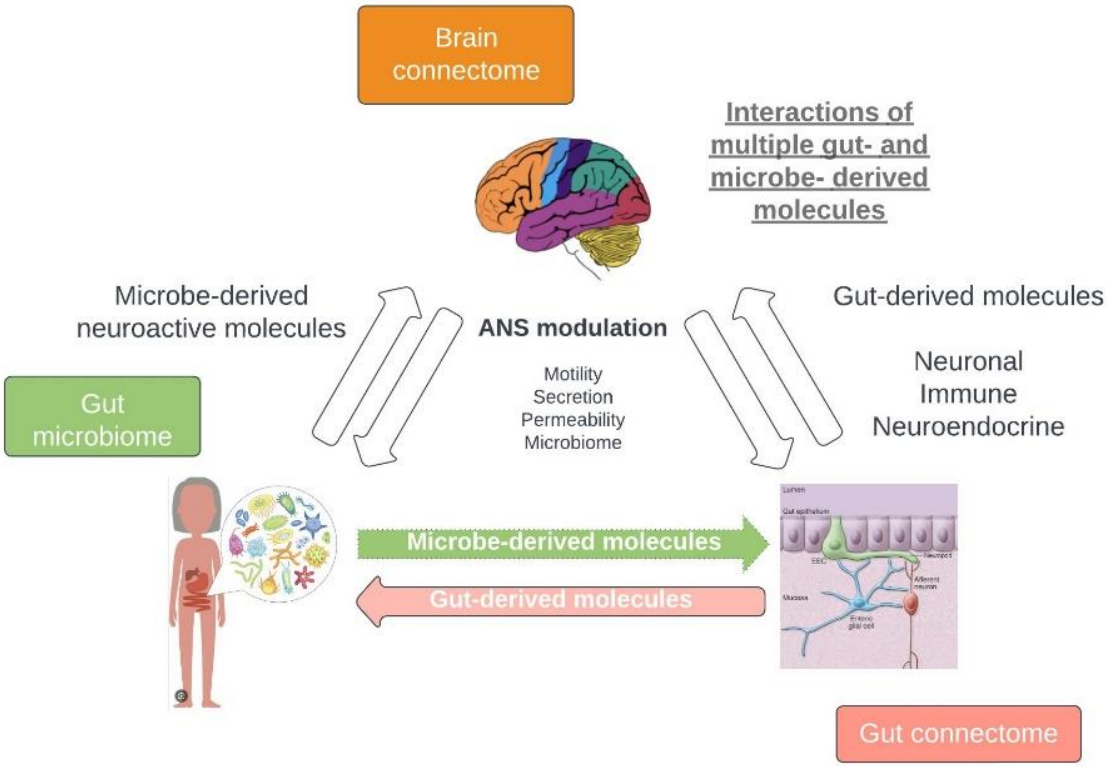

Figure 1. Interaction of multiple gut-and microbe- derived molecules [5] – The autonomic nervous system modulates motility, secretion, permeability and the microbiome as a result of signalling between Microbe-derived neuroactive molecules and Gut-derived molecules. *(ANS: Autonomic nervous system)*

Gut microbiota and brain signaling: interoception

Until recently, signals from the gut to the brain were considered to be transmitted only via fine unmyelinated vagal and sympathetic afferent fibres. Research in the microbiome sciences has concluded that interoceptive information could be transmitted to the brain via different microbes and microbial derived mediators [6].

Microbial-derived intermediates, such as short-chain fatty acids (SCFAs), secondary bile acids (2BAs), and tryptophan metabolites, interact with the central nervous system through different pathways. Some of the metabolites activate directly the enteroendocrine cells, enterochromaffin cells, and mucosal immune system to produce bottom-up signals, while other metabolites can infiltrate through the intestinal barrier and pass into the systemic circulation and could even overpass the blood-brain barrier. It is yet not entirely understood whether the concentration of these intermediates could reach a certain value that is sufficient for the activation of certain brain circuits. [6]

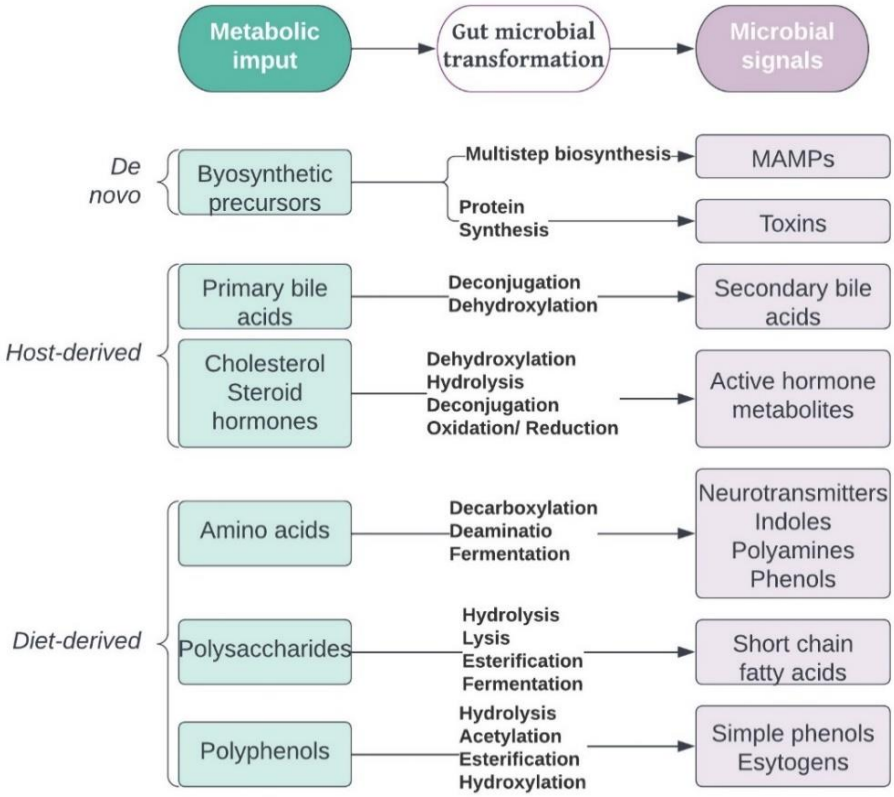

Figure 2. Means of microbial signalling [7] – Gut microbiota promotes the transformation of different molecules (De novo, Host-derived, Diet-derived) into microbial signals. Biochemical mechanisms are listed in the third column. *(MAMPs: Microbe-Associated Molecular Patterns)*

Figure 2 resumes the three main pathways through which the brain can receive information from the gut-derived molecules.

In immune signaling, parts of the microbial membranes (lipopolysaccharides, MAMPs) or even intact microorganisms, can either activate the TLRs on microglia or neurons by reaching the brain through systemic circulation or stimulate the TLRs on immune cells that are present in the gut. As a result, cytokine secretion is triggered locally as well as systemically. Host-derived molecules such as primary bile acids, cholesterol, and steroid hormones are excreted into the lumen of the small intestine and converted through different chemical interactions into neuroactive metabolites that can be reabsorbed in the circulatory system. Molecules resulting from diet, such as amino acids, polysaccharides, and polyphenols, are also modified into different molecules that can act as neurotransmitters [7].

4. Correlation between stress and gut microbiome

I. Stress

The definition of stress could be stated as the human body's reaction to the difficulties that one encounters in a certain environment. The agents that produce stress could vary in different ways. Stressors could be acute, chronic, acute on chronic, as well as repetitive acute. Depending on each person, the response to stress could differ substantially from one person to another. All things considered, stress plays a part in the susceptibility to numerous illnesses and disabilities. Thus, it is important to examine the impact of stress on human general health, as it also raises concerns for global economy.

A. Alterations in hypothalamic-pituitary-adrenal (HPA) function

Psychological and physical factors that contribute as stressors activate through different mechanisms, including hormonal changes. Firstly, they trigger the release of corticotropin-releasing hormone by activating the hypothalamic-pituitary-adrenal (HPA) axis. As a result, the adrenocorticotrophic hormone is released systemically, which then stimulates the glucocorticoid synthesis (cortisol) in the adrenal cortex [8]. Moreover, stressors induce the secretion of catecholamines (noradrenaline and adrenaline). Finally, the gastro-intestinal tract as well as the gut microbiota have the ability to react to stress and stress mediators. Bacteria in the gut are sensitive to different stress-related neurochemical mediators. By this means, the body is susceptible to bacterial infection [9]. Recent findings theorize that bacteria act as delivery vehicles for neuroactive compounds, and therefore can affect host physiology by producing neurochemicals [10].

Reversely, the influence of the gut microbiome on the HPA axis has been studied by different researchers who analyzed rodent models. Studies conducted on mice in 2014 revealed a direct connection between the microbiota and the HPA axis. There is evidence that there is an exaggerated corticosterone and adrenocorticotrophin response to stress in germ-free (GF) compared to conventionally house-specific pathogen-free (SPF) mice. GF have no commensal microbiota, which leads to a not sufficiently developed immune system [11]. In the past years, more and more information has been gathered that suggests the significant impact of the microbiota on modulating the HPA axis early in life. At birth, the stress response system is functionally immature, and it continues to develop in the postnatal period, at the same time as the colonization of the intestinal tract. In an experiment conducted on maternally separated mice, treatment with probiotics (Lactobacillus sp.) showed a significant role in reducing corticosterone levels, which had been elevated by psychological stress [12].

B. Direct influences on stress circuits

Neuronal activation of stress circuits by the microbiota has been proven to occur not only by modulating the HPA axis but also in a direct manner. Research proves that in the acute phase of the infection with *Campylobacter jejuni* in mice, induction of the neuronal activation marker cFOS was clear in the vagal sensory neurons, even when a systemic immune response was not present [13].

II. Physiological stress-exercise

High-intensity exercise has been proven to be another stressor that could determine gastro-intestinal imbalances. 30 to 90% of long-distance runners admitted in some reports that they experienced intestinal distress in relation to exercise [14]. Symptoms can vary from mild to severe and consist of nausea, vomiting, abdominal angina, and bloody diarrhea. Intensive exercise has been correlated with reduced gastro-intestinal blood flow, hyperthermia, and hypoxia, contributing to different modifications in the gut microbiome. Regarding the athletes, besides intensive workouts, another factor plays a significant role in the alterations of the gut microbiome: dietary patterns. Clarke et al. proved the effect of exercise and dietary plans on the gut microbiota [15]. Improved microbial diversity was demonstrated and positively correlated to increased protein intake and increased exercise. In comparison to two non-athletic control groups (size matched, high BMI – 30 kg/m^2 and age/gender matched), Clarke discovered lower levels of inflammatory markers and improved metabolic markers in athletes. The diversity of the microbiota was positively correlated to high protein intake and high plasma creatine kinase levels, which suggests that diet as well as high-intensity exercise influence changes in microbial diversity.

Microbial diversity was reflected by the presence of representatives of 22 phyla of bacteria, in contrast to 11 to 9 phyla in the two control groups [15].

Physical activity and fitness have the potential to affect the microbiota through various mechanisms. Intense exercise generates numerous metabolites and inflammatory mediators, whereas regular exercise and fitness can suppress basal inflammatory cytokines, indicating a feedback loop between exercise biology and host immunity [16]. Consistent physical activity has an anti-inflammatory impact that enhances the immunological profile in conditions like type 2 diabetes mellitus, coronary artery disease, peripheral arterial disease, and obesity. In animal models, repeated exercise lowers the expression of pro-inflammatory cytokines, while increasing the expression of anti-inflammatory IL-10 [8]. Furthermore, regular exercise can reduce oxidative damage to the colon in a rat model of colitis [17].

Contrasting the beneficial effect of habitual exercise, extensive exercise can negatively influence the gastrointestinal tract and its function. Due to hypoperfusion, which is present during high-intensity exercise, intestinal ischemia could result. Endotoxin translocation could also emerge as intestinal permeability increases. Recently, scientists have considered analyzing the effect of probiotic supplements on preventing gastro-intestinal symptoms such as nausea, cramping, bloating and diarrhea. Products containing Lactobacillus and/or Bifidobacterium species have been prescribed to athletes for one to six months before and/or after exercise or a competition (at varying doses of 109–1012 CFU/day). Some studies demonstrated clinical outcomes of improved upper respiratory tract illness and gastrointestinal illness, as well as immunological measures and outcomes [8].

III. Physiological Stress-Critical Illness

Similar to high intensity exercise, the intestinal hypoperfusion resulting from the redistribution of splanchnic circulation could be severe enough to cause ischemia and mucosal injury. This process could occur, in particularly in critically ill individuals. In those cases, the gut as well as the microbiome play a role in developing severe infectious complications or multiple organ dysfunction syndrome (MODS). A recent study conducted by Shimzu *et al*. analyzed the fecal pH and the presence of organic acids in patients with systemic inflammatory response syndrome (SIRS) [18]. Results suggested that these patients had reduced total anaerobic bacterial counts (especially 2–4 log fewer commensal *Bifidobacterium* and *Lactobacill us*), as well as 2 log higher potentially pathogenic
Staphylococcus and *Pseudomonas* group counts. Moreover, results showed lower concentrations of organic acids (especially Short-chain fatty acids (SCFA) acetate, propionate, and butyrate) as well as a high fecal pH in the group of critically ill patients. Alkaline and acidic pH have been associated with lower concentrations of Bacteroide and Bifidobacterium species, as well as a higher incidence of bacteremia, in comparison to the study group with normal pH. A pH greater than 6.6 has been correlated with a greater incidence of bacteremia and mortality rates. Total SCFA levels decreased with pH>6.6.

It remains unclear whether the observed changes are a result or a cause of SIRS. Although this study suggests that fecal pH could serve as a risk factor indicator, it has some limitations, such as its use of culture-based microbiota analysis and lack of specification of gastrointestinal region-specific pH levels. Research has demonstrated that the gut microbiome undergoes modifications within 6 hours of a metabolic insult and that it fails to revert to the microbial patterns observed in healthy controls [19].

Numerous meta-analyses and systematic reviews have been conducted to investigate the use of probiotics in critically ill patients. However, the outcomes of such studies may vary depending on which studies are included in the analysis. Nonetheless, it appears that the administration of probiotics to critically ill patients is associated with positive outcomes [8].

IV. Psychological Stress

Researchers developed the gut-brain axis concept at the same time that one noticed that the digestive process could be regulated by the endocrine system through different hormones. Functional Gastro-intestinal diseases such as irritable bowel syndrome and functional dyspepsia have been explained by the reciprocal action of psychological factors and altered gut physiology, the gut-brain axis. Stress that happens early in life (e.g., psychological, sexual and/or physical abuse) has been said to considerably impact the gastro-intestinal normal functions, as the period of time when the gut microbiota is developing the most is at the beginning of life.

Different studies conducted on animals suggested that psychological stress significantly influences the gut microbiome and leads to a dysfunctional gut-brain axis. Maternal separation, a model for early life stressors, has been linked to extended HPA-axis hyperactivity, anxiety-like behavior, visceral hypersensitivity, and dysfunctional cholinergic activity in the gut, as well as high permeability of the gut barrier. By attempting to restore the dysbiosis in the gut, researchers advanced probiotic treatment, that may influence the development of common functional gut disorders. In addition to this process, stress in early life has been demonstrated to promote morphological changes, such as increased number of goblet cells in the crypts of the proximal colon and subsequent amounts of mucus production and a thinner mucosal layer. Alterations in gut microbiota composition in animals that have been separated from their mothers may be caused by modifications to both gut physiology and morphology.

The reverse action of this interaction is that dysbiotic gut microbiomes cause anxiety and depression. Metabolites synthesized in the gut appear to influence brain biochemistry and behavior [8].

Clinical evidence has been published in a limited number of studies that made use of probiotics to analyze their effects on stress-related disorders. Diop et al. explored the effect of a probiotic preparation treatment of 3 weeks (Lactobacillus acidophilus and B. longum) on volunteers that experienced stress-related symptoms (anxiety, nervousness, irritability, sleeping problems, and GI disturbances) during the previous month. The probiotic seemed to provide a benefit to these individuals affected by chronic stress. A double-blind, placebo-controlled study conducted in 2016 by Kato-Kataoka showed that oral administration of L. casei strain for 8 weeks, to medical students before an academic examination contributes to the prevention of cortisol hypersecretion and stress-related symptoms. Moreover, in different research (Yang *et. al.* 2016), probiotic administration (Clostridium butyricum) to patients who were scheduled for an elective surgery (laryngectomy) ameliorated the clinical anxiety and negative effects of stress compared to placebo [20].

V. Gut-brain axis and behaviour

Different studies conducted on altered commensal intestinal microbiota, either germ-free mice, or conventionally housed animals treated with either probiotics and/or antibiotics or infected with pathogenic bacteria, show that behavioral changes occur when the gut microbiome is modified. Different strains of mice have been analyzed, as genetic background has a significant impact on the behavior of the rodents [11]. For example, there is evidence that administering low levels of pathogenic bacteria orally increased anxiety-like behaviour in the CF-1 strain, as assessed by the holeboard test [21].

Moreover, infection with the parasite Trichuris muris increases anxiety-like behavior on Balb/C and AKR strains (L/D test) [22]. On Sprague-Dawley male rodents, probiotic treatment reversed the impact of maternal separation on depressive-like behavior in rats in FST [23].

The gut microbiota can influence behavior by modulating neurotransmitter metabolism and other pathways that are yet to be fully defined via regulation of the vagus nerve. Consequently, probiotics and symbiotics may be a promising therapeutic option, either as a stand-alone or adjunct therapy to conventional treatment, for patients with irritable bowel syndrome who also have depression or anxiety [8].

Even though, treatment with probiotics in animal research has consistently proven to have a role in modulating anxiety- and depressive-like behavior, there is not enough evidence regarding their effects on psychiatric concerns in humans. Thus, in the limited number of preclinical studies, it becomes clearer that the use of probiotics might have similar effects on humans, as it has on animals [11].

5. Conclusions

To sum up, according to research, the stress effect on the gut microbiome and the gut microbiome's modulation of stress have become clearer in the past few years. As research consists of an abundance of preclinical studies, there are still not sufficient analyses on human beings. The challenge is, therefore, to translate the evidence from preclinical studies to healthy human participants in various stressful settings. Probiotics seem to be a promising intervention for stress-related disorders.

Author Contributions:

I.M.V. conceived the original draft preparation. I.M.V. was responsible for conception and design of the review. I.M.V. was responsible for the data acquisition and for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review.

Compliance with Ethics Requirements:

"The author declares no conflict of interest regarding this article".

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REVIEW

NEW MEDICAL APPROACHES IN CROHN'S DISEASE TREATMENT

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Abstract: *Crohn's disease is a lifelong, relapsing systemic inflammatory disease of unknown etiology, mainly caused by an impaired immune response, characterized by chronic inflammation of any part of the gastrointestinal tract, with an increasing incidence worldwide. This disease is associated with multiple extraintestinal manifestations and patients frequently present persistent diarrhea, abdominal pain and weight loss. It affects people of all ages, but its onset generally occurs at a young age. Several triggers have been implicated in the etiopathology of Crohn's disease, including a dysregulated immune system, an altered intestinal microbiome, genetic susceptibility and environmental factors, but the main cause of the disease still remains an enigma. Due to its debut at a young age, in most cases, a long-term and early established treatment is undoubtedly required to prevent its progression with multiple intestinal and extraintestinal complications. Nowadays, novel biologic therapies or small-molecule drugs may deeply change the innate history of this pathology and could also decrease the rate of complications and the need for surgery. In order to establish the proper diagnosis, endoscopy with the histological evaluation of biopsy specimens represents the most reliable method, even if less invasive biomarkers are being developed. Crohn's disease is a comprehensive disease and the treatment should be adapted to each patient's underlying pathogenetic mechanism.*

Keywords: *Crohn's disease, inflammatory bowel disease, diagnosis, small molecules drugs.*

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Introduction

Chron's Disease (CD) is a life-long, disabling inflammatory disorder that is often diagnosed at a young age and continues to increase in prevalence worldwide [1]. Patients with CD encounter periods of flares and remissions influenced by a complex pathogenesis, where inflammation maintains a decisive role [2]. Since its first description in modern medical literature as regional ileitis by Crohn, Ginzburg, and Oppenheimer in a case series presented at the annual meeting of the American Medical Association in 1932 [3], this pathology has persisted as an unresolved problem for both gastroenterologists and immunologists [3]. Nowadays, powerful brand-new investigative techniques are gradually leading to a better understanding of the major pathophysiological processes underlying this disease, providing this way the necessary means to access new efficient therapies [4].

Epidemiology

This disease arises from a complex interplay between genetic predisposition and environmental influence. Its prevalence has continually increased over the past 50 years,

and despite its worldwide distribution, the highest incidence has been reported in Northern Europe, the United Kingdom and North America [5]. A recent British study, published in 2020, revealed that the prevalence of Crohn's disease has increased from 220 to 400 per 100 000 and that the prevalence of inflammatory bowel disease will likely reach 1.1% by 2025 [6]. This disease has a bimodal distribution, with the onset occurring between 15 - 30 years old and 40 - 60 years old [7].

Etiology

Crohn's Disease is a chronic inflammatory condition that can affect any part of the gastrointestinal tract, from the mouth to the anus, but has a predilection to affect the distal ileum and ascending colon. Although its etiology is still unknown, the interaction between several factors, such as genetic susceptibility and the host's immune response, along with various environmental factors and the intestinal microbiome, is considered to be the main factor that may trigger the apparition of this disease [8].

Its primary patho0physiology is tissue inflammation, which is induced by an uncontrolled immunological response to luminal bacterial antigens. Immune cells like CD4 T-Cells, CD8 T-Cells, B-Cells, CD14 monocytes, and natural killers are involved in this process as they infiltrate the gut of CD patients [3]. There have been identified multiple awareness loci and many genetic factors for CD, including the NOD2 (CARD 15) gene, present on chromosome 16, which is expressed in the bacterial cell wall; people with FUT2 variants, responsible for the secretion of soluble forms of the ABO antigens, have an altered interaction with bacteria and are more prone to developing CD 8 and specific defects in the IL-10 receptor pathway and the Th17 pathway. Moreover, anomalies in the autophagy genes ATG16L1, IRGM, IL-23 receptor gene also increase the risk of CD [8,9].

Environmental factors, including cigarette smoking, a high sugar and fat income, and the so-called „Hygene hypothesis" are involved in the development of this disease. Cigarette smoking has been shown to double the risk of exacerbating CD and also to increase the risk of recurrence. A „clean" environment, which means a reduction in the body's exposure to enteric infections, may also affect the mucosal immune response, resulting in an impairment between the effector and regulatory immune responses [8,9].

Although the microbiome alterations in CD patients still remain unclear, new evidence advocates that the gut microbiota plays a crucial role in developing CD. Even if a single causative infectious agent has not been detected, there have been shown a decreased numbers of bifidobacteria and Faecalibacterium prausnitzii with a higher concentration of Bacterioides and Escherichia Coli in the samples from patients with CD compared to healthy patients. Similarly, listeria, mycobacteria, and measle-like viruses have been involved in the pathogenesis of CD, and their etiologic role is highly disputed. While the search for an infectious cause is still in progress, it seems more reasonable that its etiology is polyfactorial [8,9,10].

Clinical Features

Whereas CD most often has an insidious debut, this condition can also present as an acute toxic illness and its symptoms can be heterogeneous and subtle. One of the most common seen scenarios of Crohn's Disease is a young patient, typically complaining of abdominal pain (right lower quadrant), flatulence or bloating, diarrhea (which can include mucus and blood) or steatorrhea (in small bowel disease), fever, malabsorption, notable weight loss, even anorexia and anemia. Moreover, aphtous ulcerations of the mouth are usually observed [7]. In children, delayed puberty and a stagnation in the growth rate can also be perceived [8]. The

anus should always be examined because, in some cases, CD can become complicated by anal and perianal disease, such as abscesses, ulcers, fistulas, scarring, and cutaneous fistulas. Enteric fistulae to the bladder, abdominal wall, or vagina may also occur in 20-40% of cases, with the patient presenting pneumaturia, repetitive urinary tract infections, and feculent vaginal discharge [7,8]. Furthermore, in certain circumstances, Crohn's Disease is associated with extraintestinal features, that can affect different sites of the body, such as:

- Eyes: episcleritis, uveitis, conjunctivitis;
- Liver and biliary tree: fatty liver, chronic hepatitis, cirrhosis, gallstones, cholangitis, primary sclerosing cholangitis;
- Kidneys: nephrolithiasis, hydronephrosis, urinary tract infections;
- Joints: arthritis (spine - sacral, knee, ankles, hips, wrist, elbows), ankylosing spondylitis, arthralgia, inflammatory back pain;
- Skin disorders: erythema nodosum and pyoderma gangrenosum;
- Venous thrombosis: deep vein thrombosis, stroke or pulmonary embolism.

Diagnosis

The diagnosis of Crohn's Disease is established on the basis of endoscopy, radiological imaging, and/or histologic findings that prove the transmural inflammation of the luminal gastrointestinal tract in a patient with notable clinical manifestations (abdominal pain, chronic diarrhea). Blood and stool tests are complementary in assessing the severity and complications of Crohn's Disease, but they do not indicate the final diagnosis [11].

Routine laboratory tests may reveal anamia, which is commonly found, and it can be normocytic, normochromic, with an elevated C-reactive protein and erythrocyte sedimentation rate (ESR), hypoalbuminemia, iron and/or folate deficiency, and vitamin D deficiency [8,11]. If diarrhea is present, stool tests should always be performed. Fecal calprotectin is known to be extremely sensitive in detecting colonic inflammation, being raised in an active intestinal disease and it is also useful to predict the relapses of the disease [12]. Positive ASCA and negative pANCA antibodies represent a specific and sensitive test for Crohn's Disease, also being effective when taking into consideration the differential diagnosis with ulcerative colitis [5]. Epithelioid granulomas, transmural inflammation, "skip lesions," active and chronic inflammation, such as neutrophil-predominant cryptitis, and crypt abscesses or ulcers with chronic architectural changes are some of the classic histological signs [13].

Although the diagnosis of Crohn's Disease does not involve a single test and there is no single gold standard investigation being used, a full ileocolonoscopy with biopsies is the most universally used diagnostic investigation [5]. A solitary normal finding on ileocolonoscopy is insufficient to exclude the diagnosis of CD because approximately 27% of patients have disease situated to the terminal ileum. A relatively new and quite simple imaging technique, having the advantage of being non-invasive, is that of capsule endoscopy, which is gaining acknowledgement for small bowel exploration [5,8]. Colonoscopy or sigmoidoscopy, used in patients with severe disease, are performed when the colonic involvement is taken into consideration. As CD can also affect the upper gastrointestinal tract, upper gastrointestinal endoscopy may be required to exclude the involvement of the oesophagus or duodenum. Ultrasound scanning is a radiation-free method that can be performed at the bedside and if there is suspicion of small bowel involvement as an area affected by the disease, another non-invasive imaging technique that must be included in the local expertise is computed tomography enterography (CTE). This

technique utilizes neutral oral contrast agents and intravenous contrast medium in pursuance of identifying small bowel inflammation and visualizing the extra-enteric structures. In order to decrease the ionizing radiation that the use of the CT involves, the MRI, including MR enterography (MRE) and enteroclysis (MREC), is capable of demonstrating a wide range of pathological features of CD. F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) or radionuclide scans, such as Indium or Technetium-labeled leucocytes, may be performed in some highly specialized centers not only for initial diagnosis and for a more targeted overview of the small intestine and colonic inflammation but also for therapeutic response monitoring, suspected recurrence and to identify extraintestinal abcesses [8,14,15].

Differential Diagnosis

In order to establish a proper diagnosis of CD, especially if the pathology is uncertain, a few other differential diagnoses must be taken into consideration a few other differential diagnosis, such as ulcerative colitis, amebiasis, intestinal tuberculosis, drug-induced colitis and other conditions that are associated with small bowel fissuring ulcers [9].

CD and ulcerative colitis (UC) are two idiopathic inflammatory bowel disorders which are defined by chronic and relapsing inflammation of the bowel. Between these two conditions, there is a high degree of overlap in their clinical features, aetiopatogenesis and histological abnormalities. In UC the inflammation is generally limited to the colon, suffering a mucosal continous process, worse distally, with an increased span of involvement distal to proximal, as the disease progresses apart from minimal distal "back-wash" ileitis. The mucosa may appear reddened and inflamed and it can have a high degree of friability. The presence of granulomas is not often encountered. Moreover, UC usually includes only the mucosal layer of the bowel, and, in some cases, the superficial submucosa, unless there is fulminant colitis (toxic megacolon). In addition, the serologic findings in UC are usually positive for pANCA antibodies and negative for ASCA [8,9].

Amoebiasis is one of the diseases that must be taken into considerations in the differential diagnosis of CD. Amoebic colitis, which is a parasitic disease caused by the protozoa Entamoeba histolytica, is normally subacute and, in some cases, even asymptomatic, manifesting itself with diarrhea, abdominal pain, and rectorrhagia. Given these symptoms, infections like Escherichia Coli, Campylobacter, or Shigella should be taken into account to perform a differential diagnosis. The liver abscess is its most common extraintestinal manifestation, which is frequently associated with high fever and pain in the right hypochondrium [16].

Nonsteroidal anti-inflammatory drugs (NSAIDs), methyldopa, gold and penicillins can produce granulomatous reactions that can mimic CD. They can lead to a so-called drug-induced colitis, with several disorders that can manifest with full-thickness ulcers and resultant chronic crypt architectural remodeling. Mucosal injury can be focal or extensive, involving the entire colonic mucosa, and sometimes it can involve other parts of the gastrointestinal tract. In some cases, this type of colitis may resolve with the discontinuation of medication [9,17].

Gastrointestinal tuberculosis can occur as a primary infection or in the context of active pulmonary disease, caused by sputum ingestion or by hematogenous or lymphatic spread through infected lymphatic nodes, with the ileocecal region as its most frequently affected site. The diagnosis is often a difficult one, and it can be delayed because of its non-specific presentation. In order to detect intestinal tuberculosis, at least one of the following criteria must be encountered: acid-fast bacilli may be isolated from clinical specimens, the presence of caseating

granulomas on the histopathological investigation or the complete clinical recovery along with the healing of the gastrointestinal mucosa after 6 months, or even longer, of antituberculosis treatment [18,19].

Fissuring ulcers may also be observed in other pathologies, such as Behcet's disease, celiac sprue, or malignant lymphoma. Behcet disease is a very rare vasculitic disorder, characterized by the presence of oral and genital aphthous ulcers and uveitis, and the ability to affect small, medium and large vessels. Its diagnosis is based on clinical criteria, as there is no specific diagnostic test that can be used [9,20].

Treatment

In order to obtain proper management of CD, decisions should always be reached through a discussion between a multidisciplinary team and, of course, the patient itself. Smoking should be avoided and a healthy diet, rich in fruits and fibers, has been demonstrated to have numerous benefits [13].

1. Pharmacological treatment:

Corticosteroids: The use of oral corticosteroids such as Prednisone was once a mainstay of IBD treatment, but now it is typically reserved to induce remission in moderate to severe attacks of CD. These medications are associated with many unfavorable side effects, such as osteoporosis, high blood pressure, Cushing's disease and diabetes. Budesonide, being a glucocorticoid with a limited intestinal action and a lower systemic bioavailability, may be a better option [21].

5-aminosalicylates: In patients with inflammation limited to the rectum and sigmoid colon, this class of drugs, which contains 5-aminosalicylic acid, such as Sulfasalazine and Mesalamine, is often prescribed. These drugs are usually well tolerated, but blood tests should be performed every few months, in order to monitor hepatic and renal function [8,21].

Immunosuppresive drugs: These agents have variable efficacy and different indications. Azathioprine, methotrexate, 6-mercaptopurine, tacrolimus and cyclosporin are the most frequently used, representing conventional maintenance remission therapy. These medications have the ability to reduce inflammation in the gastrointestinal tract by suppressing the immune system. Due to their numerous side effects (liver injury, bone marrow suppression), their usage is declining, and sometimes it is more preferable to use them in lower doses with biologic medications for a better enhancement of treatment [22].

Biologic therapies: Although this class of medications was historically reserved for the treatment of severe cases, the modern approach to CD nowadays suggests that they should be used as a first-line approach, becoming the standard first-line therapy in the care of patients. With many ongoing trials and newly discovered agents, this therapeutic approach is promising and proving to be a rapidly developing area [23].

1. Anti-TNF alpha therapies: Notwithstanding the fact that the exact mechanism of action is still not fully defined, these medications block the tumor necrosis factor (TNF) and induce cell apoptosis. Available anti-TNF agents consist of infliximab, adalimumab and certolizumab pegol, and they have led to improved rates of both response and remission in patients. These drugs can induce anti-drug antibodies and can become less effective if they are not used on a regular basis [24].

2. Anti-IL-12/23: These therapies have the ultimate goal of treating Crohn's Disease while minimizing the side effects that commonly appear and reducing intestinal inflammation by inhibiting specific pro-inflammatory proteins, such as interleukin-12 and interleukin-23. They are currently used to treat moderately to severley active CD in those patients who are not responding enough to anti-TNF-alpha agents. Ustekinumab is already FDA-approved to treat CD and it has

proven its efficiency. Risankizumab was FDA-approved in June 2022 to treat patients with moderately to severely active Crohn's disease. Three other monoclonal antibodies, mirikizumab, brazikumab and guselkumab are currently in advanced clinical trials for CD [25].

3. Anti-Integrin: Vedolizumab (Entyvio), which is an anti- α4β7 integrin therapy, reduces leucocyte recruitment to the inflamed intestine. It is used both in induction and maintenance therapy. This drug may be administered as IV infusions or subcutaneous injections. It was approved worldwide as an intravenous [IV] 300 mg formulation to treat moderately to severely active CD and in 2020 a SC formulation of Vedolizumab [Vedolizumab SC] was developed to provide an alternative route of Vedolizumab administration for use in UC and CD in Europe, Canada and Australia as maintenance therapy (108 mg every 2 weeks) [8,26].

Small molecule drugs (SMDs): Despite the fact that the anti-TNF-alpha, anti-integrin, and anti-IL12/13 medications have substantially improved the management of Crohn's Disease, because of the anti-drug antibody formation, their effectiveness is not always as expected. In order to tackle this issue, therapy with SMDs, which are orally administered, has been newly established. These medications use a broad range of novel pharmacological pathways and they may even lack of immunogenity [23,27].

1. JAK inhibitors: These therapies interfere with the activity of Janus kinases (JAK), being targeted therapies that work on the body's inflamatory immune response. Upadacitinib is the first oral agent and distinguishes itself from the injectable biological therapies used in CD, by being well-tolerated both in the induction and maintenance phases, representing a promising agent. It was FDA-approved in March 2022 and it was associated with superior endoscopic outcomes at 12 weeks and 1 year compared with placebo among people with moderately to severely active CD, and it was efficient in patients with previous failure of biologic therapy. Upadacitinib has the main advantages of a single oral daily administration, a fast onset and a low risk of immunogenicity [28,29].

2. S1P receptor modulators: This class of medication blocks the receptor of a signaling fat molecule called S1P and its effect is to reduce inflammation and the immune response. Ozanimod, an oral agonist of the S1P receptor subtypes 1 and 5 decreases the number of circulating activated lymphocyteswas and was approved in May 2021 for the treatment of moderate to severe UC. Ponesimod and Etrasimod, which are currently in phase 3 trials for the treatment of IBD, are another two selective S1P modulators that are on the verge of proving their effectiveness in IBD treatment [30].

3. Anti-sense oligodeoxynucleotide to SMAD7- Monsergen, an oral anti-sense oligonucleotide, that restores TGF-beta 1 signaling and decreases the output of pro-inflammatory markers, is another promising new treatment for CD, phase 3 studies ongoing [8,31].

Stem cell therapy, mostly including hematopoietic stem cell therapy and mesenchymal stem cell therapy, has shown the potential to improve the clinical disease activity of patients when pharmacological treatments are not effective. Nevertheless, this type of therapy is still in the research stage. Stem cell therapy, an emerging therapy for IBD, may have the ability to both repair and regenerate the damaged tissue and may ease the mucosal inflammation. In this manner, this therapy can help to enhance symptoms such as abdominal pain and diarrhea and may even hinder the disease's advancement. Darvadstrocel (Cx601), the only commercially available mesencymal stem cell preparation approved by The United States Food and Drug Administration (FDA), can be used in the treatment of CD, complicated by perianal fistula [32,33].

Surgical management: While pharmacological treatment remains the first

treatment option, almost 80% of people with Crohn's disease will eventually require surgery at some point in their lives. As recurrence is almost unavoidable, surgery should be performed only under special medical conditions and minimal resections are preferred. Some common types of surgeries implemented in Crohn's Disease include:

- Stricturoplasty, where some strictures, which are complications commonly encountered, can be widened;
- Fistula removal;
- Colectomy or panproctocolectomy with ileostomy, when the disease involves the colon; the ileostomy may be temporary, in order to reduce the inflammation, or permanent;
- Bowel resection;
- Abcess drainage [8].
-

Prognosis

The life expectancy in those with Crohn's Disease is mildly reduced compared to the general population, due to the risk of developing malignancies and multiple complications of different organs (liver, genitourinary tract, biliary tract). Almost half of the patients will need a surgical resection within the first 5 years of the disease, and pharmacological treatment should be initiated in the first years of the disease. An extensive small bowel disease, young adults (<20 years old), serious ulcerations, and complicated perianal disease represent some of the poor prognosis factors [8,34].

Conclusions

The management of this disease requires a multi-speciality team, including gastroenterologists, primary care physicians, surgeons, oncologists and many others. Despite the fact that there is no cure for Crohn's Disease, nowadays the overall mortality has decreased steadily, owing to new pharmacological therapies, that have greatly expanded the treatment options available for the management of this disease.

Author Contributions:

M.I.M. conceived the original draft preparation. M.I.M. was responsible for conception and design of the review. M.I.M. was responsible for the data acquisition and for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review. All authors contributed to the critical revision of the manuscript for valuable intellectual content.

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REVIEW

DEFICIENCY AND TOXICITY OF VITAMINS

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Abstract: Vitamins are substances necessary to sustain life, with many functions. Vitamins must be obtained from food, as they are either not made in the body at all or are not made in sufficient quantities for growth, vitality and wellbeing. Lack of a particular vitamin can lead to incomplete metabolism, fatigue and other important health problems. Deficiency of a vitamin causes symptoms which can be cured by that vitamin. Large doses of vitamins may slow or ever reverse diseases such as cancer, osteoporosis, nerve degeneration and heart disease.

Keywords: vitamins, deficiency, excess, water-soluble vitamins, fat-soluble vitamins.

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Abreviations: ACP-acyl carrier protein CRP- C-reactive protein; DRI-dietary reference intake; GPX-3-Glutathione peroxidase 3; G6PD-Glucose-6-phosphate dehydrogenase; EAR-estimated average requirement; Holo-TC-holo-transcobalamin; HPLC-high-pressure liquid chromatography; MMA-methylmalonic acid; NAD-nicotinamide adenine dinucleotide; RDA-reccomended dietary allowance; ThDP-thiamine diphosphate; VKDp -vitamin K dependent-proteins.

INTRODUCTION

Vitamins are substances which are necessary to sustain life. They are divided in water-soluble vitamins (B-complex) and fat-soluble vitamins (vitamin A, vitamin D, vitamin K and vitamin E). Vitamins are essential for the normal function of human body metabolism. They play a role in metabolism enabling the body to use other essential nutrients such as carbohydrates, fats, proteins and minerals. Individual vitamins have specific functions which vary widely and can overlap. They are involved in growth and the maintenance of health and are important for a normal appetite, in digestion and resistance to bacterial infections. It is important to understand that vitamins are not substitutes for food.

THIAMINE (VITAMIN B1)

Thiamine is a water-soluble vitamin essential for carbohydrate metabolism and energy metabolism [1]. Body stores of thiamine are limited and dependent on dietary intake. There are five natural thiamine phosphate derivatives.

The absorption of vitamin B1 occurs in the jejunum and ileum and it can be inhibited by

alcohol consumption and folate deficiency. The EAR for women and men are 0.9-1 mg/day [2], [3]. Enteral nutrition should provide 1.5-3 mg/day and parenteral nutrition at least 2.5 mg/day of thiamine.

The main sources of vitamin B1 are whole grains, legumes, meats, nuts and fortified foods. Thiamine reserves are depleted in 20 days of inadequate oral intake. Patients at risk of deficiency include malnutrition, poor oral intake and chronic alcohol consumption, malignancies and pregnancy. Reduced gastrointestinal absorption and increased gastrointestinal or renal losses and obesity pre-bariatric surgery should be considered [3]. Critical illness like sepsis and major trauma are associated with thiamine deficiency or depletion.

Thiamine status may be determined using indirect and direct methods. Erythrocyte transketolase activity is an indirect method, while direct methods include the quantification of ThDP, the coenzyme of thiamine in whole blood or red blood cells.

Thiamine deficiency symptoms include neurological, psychiatric and cardiovascular systems [1], [4]. Apathy, decrease in short-term memory, confusion and irritability to cognitive deficits and Wernicke-Korsakoff encephalopathy, optic neuropathy, Leigh's disease, African Seasonal Ataxia and central pontine myelinolysis [5]. Vitamin B1 can be administred by oral, enteral and intravenous routes and is cheap all over the world. There is no toxicity of thiamine.

RIBOFLAVIN (VITAMIN B2)

Riboflavin is also a water-soluble vitamin and is involved in redox reactions, antioxidant functions, metabolism of the other B vitamins and energy production. It has several immunomodulatory effects.

Absorption takes place in the proximal small intestine and it is also produced by the microflora of the large intestine. Riboflavin is excreted in the urine and it is not stored in the body [6].

Main sources: enriched and fortified grains, cereals and bakery products, meats, fatty fish and eggs [7]. The RDA is 1,3 mg/day in men, 1.1 mg/day in women and 1.4 mg and 1.6 mg during pregnancy and lactation. The riboflavin status can be assessed by the glutathione reductase activity in red blood cells [6].

Deficiency of riboflavin is manifested by oral lesions, seborrheic dermatitis of the face, trunk and scrotum, ocular symptoms and normochromic, normocytic anemia and marrow aplasia. Patients at risk are those with malabsorption, thyroid dysfunction, diabetes, alcoholism and in pregnancy, lactation, patients with surgery, trauma, burs and patients on psychotropic drugs, tricyclic antidepressants or barbiturates. Riboflavin deficiency is associated with pyridoxine, folate and niacin deficiency [6], [7]. Toxicity of vitamin B2 is rare.

NIACIN (VITAMIN B3)

The metabolically active form of niacin is the coenzyme NAD. Niacin helps to convert nutrients into energy, create cholesterol and fats, create and repair DNA. Niacin can be synthesized from the amino acid tryptophan in the liver, a pathway which also requires thiamine, riboflavin and pyridoxine. Blood or tissue NAD can be used for the assessment of niacin [2], [7].

Main sources: processed foods, meat, poultry, red fish, nuts, legumes and seeds. Adolescent and adults intake is 16 mg/day (males), 14 mg/day (females) and 18 mg/day (pregnant women) [8],[9]. Enteral nutrition should provide 18-40 mg/day and parenteral nutrition should provide 40 mg/day of this vitamin.

Primary niacin deficiency occurs on a corn-based diet and in general malnutrition. Secondary causes include chronic alcoholism and prolonged diarrhea. "Pellagra" or "the three D disease" is characterized by diarrhea, dermatitis and dementia. Other causes of deficiency are inadequate oral intake, defective tryptophan absorption, carcinoid

tumors and chemotherapeutic treatments [10], [11], [12]. A prospective population-based study suggested that dietary niacin protects against Alzheimer disease and age-related cognitive decline [13].

Flushing in the face, arms and chest and hepatotoxicity are symptoms of toxicity. Hepatitis can be produces by energy drinks that contain large quantities of niacin [14].

PANTOTHENIC ACID (VITAMIN B5)

Pantothenic acid is part of the coenzyme A and acyl carrier protein. It is involved in oxidative respiration, lipid metabolism and synthesis of steroids. 85% of vitamin B5 exists as derivatives such as coenzyme A phosphopantetheine and ACP which are converted to pantothenic acid by pancreatic enzymes [15]. It is absorbed along the small intestine.

The DRI is 5 mg/day and the needs increase to 6-7 mg/day for pregnant and lactating women [2]. Enteral nutrition should provide 5 mg/day and parenteral nutrition should provide 15 mg/day of vitamin B5 along with other B-vitamins.

The sources are fortified cereals, organ meats, beef, chicken, avocado, nuts and milk products.

Deficiency of pantothenic acid was observed only in conditions of severe malnutrition. Symptoms of deficiency include fatigability, frequent upper respiratory infections, but severe deficiency may lead to headache, extreme tiredness, irritability, sleeping problems, nausea and vomiting [16]. Cerebral pantothenate deficiency is a newly identified metabolic defect in Huntington and Alzheimer diseases [17]. In context of neurological symptoms, pantothenic acid blood determination should be performed. Derivative of pantothenic acid is often used as a source of vitamin in vitamin supplements. Calcium pantothenate is used in dietary supplements because, as a salt, is more stable than pantothenic acid. Pantothenic acid supplementation might reduce lipid levels in patients with hyperlipidemia because of the role in triglygeride syntehesis and lipoprotein metabolism.

No toxicity of vitamin B5 has been reported.

PYRIDOXINE (VITAMIN B6)

Pyridoxal phosphate (PLP) is the biologically active form of pyridoxine which serves as coenzyme for more than 160 enzymatic reactions (transamination, racemization, decarboxylation). The most important role of vitamin B6 is related to the biosynthesis as the degradation of amino acids, is central to transamination reactions. Other functions are gluconeogenesis, steroid receptor binding and heme biosynthesis [19].

Absorption of pyridoxine occurs in the small bowel. The DRI is 1.3-1.7 mg/day for men and women. The need can reach 2 mg/day for pregnant women. Normal values of PLP are 5-50 μg/L [2].

Diet is the only source of vitamin B6. Sources of pyridoxine are meat, whole grains and potatoes. Plasma levels of pyridoxal 5-phosphate correlate with pyridoxine intake and body stores and is recognized as a status biomarker. PLP may be determined in plasma, serum and erythrocytes [20].

Inflammation leads to a fall in plasma PLP, but minimally affects red blood cell concentrations. Symptoms of pyridoxine deficiency include seborrheic dermatitis with cheilosis and glossitis, microcytic anemia, confusion, depression and angular stomatitis [21]. Causes of deficiency are isoniazid therapy, HIV infection therapy and treatment, alcoholic hepatitis, migraine attacks and thymoglobulin immunosuppression for organ transplantation. Pyridoxine-dependent epilepsy is a rare autosomal recessive epileptic encephalopathy caused by antiquitin deficiency. Population at risk of deficiency include alcoholics, renal dialysis patients, the elderly, critical illness, pregnancy and people receiving medical therapies that inhibit vitamin activity [18], [22], [23], [24].

Sensory neuropathy with ataxia or areflexia, impaired cutaneous and deep

sensations and dermatological lesions are clinical signs of excess pyridoxine. Long-term doses as low as 100 mg/day have been associated with spinal cord affections [25]. In context of isoniazid overdose or glycol poisoning, high dose of pyridoxine should be used as part of the treatment.

BIOTIN (VITAMIN B7)

Also, a water-soluble vitamin, biotin can be found in all cells of human body. Main functions of this vitamin include: metabolism of the fatty acids, glucose and amino acids as it is a cofactor for five carboxylases critical for their metabolism. Biotin is an essential vitamin for normal fetal development [26].

Healthy adults need 40 μg/day and lactating women 45 μg/day. The Institute of Medicine recommends 30 μg/day plus additional 5 μg/day for lactating women. Vitamin B7 can be administered by oral and intravenous routes. Enteral nutrition should provide 30 μg/day and parenteral nutrition 60 μg/day of vitamin B7 [26], [27].

Biotin sources: egg yolks, milk, organ meats and multivitamin supplements. For vegans main sources of biotin are peanuts, avocado, sweet potatoes and tomatoes. Biotin status has to be determined by the direct measure of blood and urine level of biotin and should be completed by the determination of biotindinase activity.

Deficiency of biotin is rare, but when is present leads to dermal and neurological complications. Causes of deficiency include chronic alcohol consumption, malabsorption in Chron's disease, smoking and pregnancy [28], [29]. Anticonvulsant therapy can interfere with the absorption of vitamin B7 and increase needs, but more recent data suggest that there is no concern about valproate and carbamazepine [30].

No adverse effects of biotin pharmacological doses have been shown.

FOLATE AND FOLIC ACID (VITAMIN B9)

The biologically active folate forms are folinic acid and 5-methyltetrahydrofolate (5-MTHF). Folate has an important role as cofactor in the metabolism of nucleic acid precursors and several amino acids [31].

Absorption of folates occurs in the duodenum and jejunum in a pH-dependent carrier-mediated process. Folic acid is manufactured synthetically and is available in supplements or fortified foods: it is converted in the body into folate [32].

Sources of folate: egg, nuts, whole grain products and green vegetables. The DRI varies from 250 to 400 μg/day. Pregnant and lactating women needs are about twice as high [33], [34]. Folic acid may be administered orally, enteral, subcutaneous, intravenous and intramuscular. Enteral nutrition should provide 330-400 μg/day and parenteral nutrition 400-600 μg/day of folic acid. Folate status shall be assessed in plasma or serum (short-term status) or red blood cells (long-term-status). Red blood cells folate level is a sensitive marker of long-term folate status because it informs about folate accumulation during red cell erythropoiesis as well as tissue folate stores.

Folate deficiency overlap with cobalamin deficiency. Symptoms include glossitis, angular stomatitis, oral ulcers, depression, insomnia, anorexia and fatigue. Deficiency of both vitamins causes megaloblastic anemia [35]. Causes of folate deficiency may be inadequate dietary intake (poverty poor nutrition), intestinal malabsorption (celiac disease, chronic intestinal failure), increased needs (pregnancy and lactation, neoplastic diseases, renal dialysis) and antifolate drugs (sulfasalazine, methotrexate, anticonvulsants). In case of dietary deficiency or chronic hemodialysis 1-5 mg folic acid/day may be given orally.

Folic acid has proliferative effects so it might increase cancer risk and progression. It is said that folic acid can cause insulin resistance in children, mask a vitamin B12

deficiency and be hepatotoxic [36]. Excess folic acid is excreted in the urine.

1. COBALAMIN (VITAMIN B12)

Cobalamin is a water-soluble vitamin synthesized by fungi and microorganism and in the stomach of ruminant animals dependent on soil cobalt content. Humans are totally dependent upon animal sources of fortification [37], [38].

Cobalamin absorption has several steps, and it requires normal stomach, pancreas and small intestine function. Vitamin B12 is a cofactor for two enzymes in humans: methionine synthase in methyl transfer from methyl tetrahydrofolate to form methionine from homocysteine and methyl malonyl-coA mutase in synthesis of the citric acid cycle intermediate succinyl coA [38].

The main sources of vitamin B12 are ruminant meat, organs, milk, fish, fortified cereals and nutritional yeast. The DRI for adults is 2,4 μg/day. In pregnancy the needs are 5 μg/day and in lactation 4,5 μg/day. In adults, vitamin B12 reserves are approximately 2500 μg and it may last for 12-36 months without sufficient intake [89]. Enteral nutrition should provide 2.4 μg/day and parenteral nutrition 5 μg/day of cobalamin.

The main cause of low serum cobalamin in younger adults is inadequate intake because of low consumption of animal-sources [39]. The most common causes of deficiency are an autoimmune condition as pernicious anemia, food-bound cobalamin malabsorption and chronic atrophic gastritis. Primarily, the manifestations are haematological and neuropsychiatric deterioration. Clinical symptoms include macrocytosis, reticulocytosis, anemia with pallor, fatigue, tachycardia, peripheral neuropathy, paresthesia, tingling, vertigo, ataxia, irritability, psychosis, depression, confusion, cognitive decline, dementia, glossitis, and weakness. It is very important that vitamin B12 deficiency be excluded in all patients who present with anemia or isolated macrocytosis, established diagnosis of polyneuropathies, neurodegenerative diseases or psychosis.

There is no upper toxicity limit for cobalamin and no reports of acute toxicity, but cobalamin excess with high blood levels has been observed in alcoholism, liver disease and cancer [40].

Adults at risk or suspected of cobalamin deficiency should be screened with the combination of at least two markers (holo-TC, MMA) with serum cobalamin as a replacement.

VITAMIN A

There are two different active metabolites of vitamin A: retinoic acid and retinal which are responsible for vision and reproductive function [41]. It also has an important role in the immune system. Retinol binding protein is a negative acute phase protein which leads to a fall serum retinol. Inflammation also reduces absorption of vitamin A and increases requirement and urinary loss which together may contribute to the development of vitamin A deficiency.

Main sources of vitamin A are liver, meat, fish, cheese and butter, spinach, broccoli and mango. 90% or more of whole-body vitamin A is stored in the liver. In case of vitamin A intake below recommendation, the liver stores are sufficient to maintain functions for about 6 months.

The best measurements are the concentration of vitamin A in the liver or total body retinol.

Vitamin A deficiency is a public health problem in most developing countries due to malnutrition, especially in children and pregnant women. Clinical signs and symptoms are increased susceptibility to infections, especially of the respiratory tract, night blindness due to insufficient rhodopsin synthesis and xerophthalmia. Keratomalacia with expansion to iris and lens area leading to xerophthalmia and finally blindness is the worst complication of vitamin A deficiency.

Inflammation leads to decreased serum/plasma retinol concentration [42], [43].

Acute toxicity appear when high quantities of natural vitamin A are ingested within a few hours or days and it is manifested with increased intracranial pressure, nausea and headaches. Chronic toxicity may leads to hepatotoxic effects [44], [45]. There is no treatment of vitamin A toxicity. Retinoids (retinol, retinal, retinoic acid and related compounds) in high concentrations are teratogenic [46].

VITAMIN C

Vitamin C has numerous functions which are all based on electron donation. It is an important cofactor/ co substrate for the biosynthesis of neurotransmitters, cortisol, peptide hormones and collagen. Vitamin C can limit the inflammatory response and ischemia-reperfusion injury, improve host defense, wound healing and mood and has a role in pain reduction [47], [48], [49].

Humans are dependent on dietary intake of fruits and vegetables because they are unable to synthesize vitamin C. The DRI for vitamin C is 90-100 mg/day. Enteral nutrition should provide at least 100 mg/day and parenteral nutrition should provide 100-200 mg/day of vitamin C.

Assessment of vitamin C status can be determined from its concentration in plasma or leukocytes. Vitamin C includes L-ascorbic acid (AA) and its oxidation product dehydroascorbic acid (DHAA). Plasma vitamin C analysis is the preferred option for status assessment and serum determination should be avoided.

Normal plasma vitamin C levels are defined as >23 μmol/L. Hypovitaminosis C has been defined as plasma levels less than 23 μmol/L and vitamin C deficiency as less than 11 μmol/L [50]. Vitamin C plasma levels decline rapidly with inflammation making interpretation difficult. It may be administered oral, intramuscular, intravenous or subcutaneous. For intravenous administration the drug should be diluted with normal saline or glucose to minimize adverse reactions.

Clinical conditions with increased inflammation and oxidative stress such as sepsis, trauma, cardiac arrest, major surgery and burns are associated with high risk of depletion. In critically ill patients, low plasma vitamin C concentrations are associated with severity of oxidative stress, organ failure and mortality. Chronic depletion appears in patients after bariatric surgery, alcoholism, chronic dialysis, smoking, chronic inflammation. Clinical symptoms are lassitude, anemia, poor wound healing, myalgia and bone pain, edema, loose teeth, bulging eyes, dry hair and shortness of breath [51], [52].

Vitamin C supplementation is contraindicated in blood disorders like thalassemia, sickle cell disease and hemochromatosis. Symptomatology includes urinary calcium oxalate crystallization, renal stone formation and nephropathy, factitious hyperglycemia and hemolysis in patients with G6PD deficiency [53].

VITAMIN D (25-HYDROXIVITAMIN D)

Vitamin D is a steroid hormone precursor. Cutaneous endogenous production is possible from cholesterol with UV-B exposure, explaining the strong seasonal variations in vitamin D levels. Vitamin D supply is also possible by nutrition, but it does not cover the needs. It has several effects on many organs including bones, muscle, heart and nervous system.

The recommended daily oral intakes of vitamin D is 600-800 IU in adults and 1500-4000 IU in patients “at risk for vitamin D deficiency”. It may be administered by oral, enteral intravenous or intramuscular route [54], [55].

The valid marker for vitamin D assessment is serum/plasma concentration of total 35-hydroxyvitamin D, the sum of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2. There is no ideal time to measure the status of vitamin D.

Plasma levels of this vitamin are significantly reduced in the context of inflammation: in presence of CRP>40 mg/L, nearly all values are below reference ranges, complicating the interpretation [56].

The classic vitamin D deficiency syndrome is rickets in children and osteomalacia in adults. Vitamin D deficiency is defined by a plasma concentration of <50 nmol/L and severe deficiency by a plasma concentration <30 nmol/L. Patients at risk are those with severe kidney or liver dysfunction and chronically il patients.

Intoxication is rare, but it has been described and the symptoms include hypercalcemia, hypercalciuria, dizziness and renal failure [57].

2. VITAMIN E (ALPHA-TOCOPHEROL)

Alpha-tocopherol, the natural vitamin E with the highest biological activity is a component of all biological membranes and is the most important lipid-soluble antioxidant. It most important role is to protect membrane lipids, lipoproteins and depot fats from lipid peroxidation [58].

The DRI for vitamin E for adult men and women is 12 mg/day, 15 mg/day in pregnancy and 19 mg/day in lactation. Enteral nutrition should provide at least 15 mg/day and parenteral nutrition should provide at least 9 mg/day of alpha-tocopherol [59].

Vitamin E status is determined by the quantification of alpha-tocopherol in blood plasma or serum collected into plain, gel separator, heparin or EDTA tubes followed by HPLC coupled with ultraviolete or fluorescence detection. Plasma vitamin E is age-dependent and in children and young people significantly lower than for adults. Blood levels of vitamin C are little affected by inflammation, nevertheless the blood concentrations become less interpretable with CRP values >80 mg/L. Alpha-tocopherol is degraded in inflammatory and organ failure conditions due to increased lipid peroxidation [20.

Vitamin E deficiency is rare and it may appear in severe malnutrition. Genetic causes of vitamin E deficiency are abetalipoproteinemia with disturbance of absorption and the absence of the alpha-tocopherol transfer protein with distribution restrictions [60]. In adults with fat malabsorption, early vitamin E inadequate is generally asymptomatic. Neurological symptoms are associated with balance and coordination disorders, peripheral neuropathy and muscle weakness. In case of long-standing fat malabsorption, vitamin E supplementation improves neurological symptoms after a few months, following normalization of vitamin E status [61].

Vitamin E should be determined when there is clinical suspicion of vitamin E deficiency that include cystic fibrosis, abetalipoproteinemia and thrombotic thrombocytopenic purpura. In case of absence of clinical signs of deficiency there is no indication to measure vitamin E status.

VITAMIN K (PHYLLOQUINONE)

Vitamin K includes vitamers known as vitamin K1 (phylloquinone) and vitamin K2 (menaquinones). Vitamin K1 is produced by the plants and vitamin K2 is synthetized by human intestinal microbiota. Vitamin K includes a group of lipid-soluble molecules that possess carboxylase enzyme cofactor activity necessary for the activation of vitamin K dependent-proteins (VKDp). These include the coagulation factor proteins C, S, M, Z, factors VII, IX, X and prothrombin [62].

Main sources are leafy greens, cruciferous vegetables, asparagus, prunes and peas. Many intestinal bacteria, including E. coli synthesize vitamin K2, but no vitamin K1. Enteral nutrition should provide at least 120 μg/day and parenteral nutrition should provide 150 μg/day of vitamin K.

Fat malabsorption, malnutrition, antibiotic and anticoagulant treatments are the most common causes of vitamin K deficiency. Vitamin K can contribute to significant

bleeding, poor bone development, osteoporosis and increased cardiovascular disease. Clinically significant bleeding has mainly been reported in newborns and extremely inadequate intake or malabsorption syndromes.

Vitamin K1 and K2 are not associated with toxicity. Rare anaphylactoid reactions with bronchospasm and cardiac arrest after intravenous vitamin K1 administrated for anticoagulation reversal have been reported [63]. The synthetic vitamin K3 is very toxic and could cause jaundice, hyperbilirubinemia, hemolytic anemia and kernictus in infants.

Vitamin K status should be determined by a combination of biomarkers with dietary intake, as there is no agreed standard.

Author Contributions:

V.M.M. conceived the original draft preparation. V.M.M., R.I.D., A.I.N. and L.B.G. were responsible for conception and design of the review. V.M.M., L.B.G., A.I.N. and R.I.D. were responsible for the data acquisition. V.M.M. was responsible for the collection and assembly of the article/published data, and their inclusion and interpretation in this review. All authors contributed to the critical revision of the manuscript for valuable intellectual content. All authors have read and agreed with the final version of the manuscript.

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CASE REPORT

ADENOSQUAMOUS LUNG CARCINOMA COMPLICATED WITH MARANTIC ENDOCARDITIS AND CHRONIC DISSEMINATED INTRAVASCULAR COAGULATION

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ABSTRACT

Background*: Adenosquamous lung carcinoma is a relatively rare subtype of non-small-cell lung cancer that contains both adenocarcinoma (ADC) and squamous cell carcinoma (SCC) components. It is difficult to reach a proper diagnosis before surgery, with the histopathological examination of the resected specimen being the method of choice. A supplementary immunohistochemistry examination of the sample is needed. In order to initiate targeted treatment, molecular testing is mandatory.*

Case presentation*: A 47-year-old female, smoker (15 packs-year), without pathological personal history, presented for fatigue and moderate bilateral leg edema for about 3 months. The blood tests showed severe microcytic, hypochromic anemia and chronic disseminated intravascular coagulation. Computed tomography (CT) scan revealed a tumor in the right basal pleura with secondary pleural effusion, multiple lymphadenopathies, disseminated in the mediastinum, abdominal and pelvic cavity. Two other tumors were noticed, one in the right breast (7 mm) and the other in the vesicouterine pouch (7/10 mm). Prior to lung biopsy, a transthoracic and then transesophageal echocardiography were performed, highlighting the presence of a band on the aortic valve, suggesting marantic endocarditis. Tumor markers were also elevated. In evolution, the patient became bradypsychic, with head CT showing a brain metastasis in the left high-parietal region. Immunohistochemistry examination of the biopsy sample suggested a adenosquamous lung carcinoma.*

Conclusion*: We reported the diagnostic path of a rare subtype of lung cancer in a young female without known comorbidities, with an atypical presentation - multiple extrapulmonary non-metastatic manifestations: metabolic etiology - weight loss, fatigue; vascular and hematological etiology – marantic endocarditis (a very rare complication), severe microcytic, hypochromic anemia and chronic disseminated intravascular coagulation; neurological etiology – peripheral sensorimotor neuropathy of the right arm. A tissue biopsy was performed from the most accessible region – 1/3 inferior right thorax, posterior axillary line. The particularity of the adenosquamous lung carcinoma in this case lies in multiple metastases in less common sites (breast, vesicouterine pouch and probably kidney).*

Keywords: lung carcinoma, disseminated intravascular coagulation, endocarditis.

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INTRODUCTION

According to the International Agency for Research on Cancer - Global Cancer Observatory (IARC – GCO), lung cancer ranks second in incidence (after breast cancer) and first in mortality in both sexes. In addition, lung cancer has overtaken prostate cancer, ranking first in incidence among men in 2020. In women, lung cancer ranks third in incidence after breast and colorectal cancer, and second in mortality after breast cancer [1].

Men are twice as likely to be diagnosed with lung cancer, given tobacco use, although women may be more susceptible due to the higher amount of epidermal growth factor receptor mutations and the effects of estrogen. Lung cancer is divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) –the latter having a higher incidence (85%) and being further divided into: adenocarcinoma (ADC), squamous cell carcinoma (SCC) and neuroendocrine cancers [2].

CASE PRESENTATION

We report the case of a 47-year-old female, smoker (15 pack-year), without known comorbidities who came to the emergency room for progressively worsened fatigue that started 3 months before presentation, bilious vomiting that occurred three days before presentation, moderate bilateral leg edema and inappetence. The patient also reports unintentional weight loss of approximately 10 kg in about a year.

At presentation, the patient had an altered general state and pale skin. She had hypertension (blood pressure = 169/75 mmHg) and peripheral edema in both legs. Respiratory auscultation revealed the presence of sibilant rales in the left basal lung without the need for oxygen support (saturation level of 97% while breathing room air). The patient had reported pain in the right lumbar area with a positive Giordano's sign.

She was admitted to the internal medicine clinic presenting mild leukocytosis with neutrophilia, inflammatory syndrome (C-reactive protein (CRP) 73.55 mg/L), severe microcytic, hypochromic anemia – hemoglobin 2.9 g/dL – for which she received 2 IU of RBC transfusion isogroup, isoRh, iron deficiency (1.81 umol/L), ferritin – untested, hypoalbuminemia (2.8 g/dL), mild hyperglycemia (146 mg/dL) and chronic disseminated intravascular coagulation (high D-dimer 9759 ng/mL, low fibrinogen 103 mg/dL, prolonged prothrombin time 18.6 sec, but normal platelet count). The electrocardiogram was normal. Moderate biliary residue, 3 mm cholesterol polyp, alithiasis and 50 mm liquid in the right costophrenic sinus were seen at abdominal ultrasonography. Given the symptomatology, a chest X-ray (Figure 1) was performed and revealed infra hilar alveolar opacities on the right side and pleural effusion in moderate quantity in the same region.

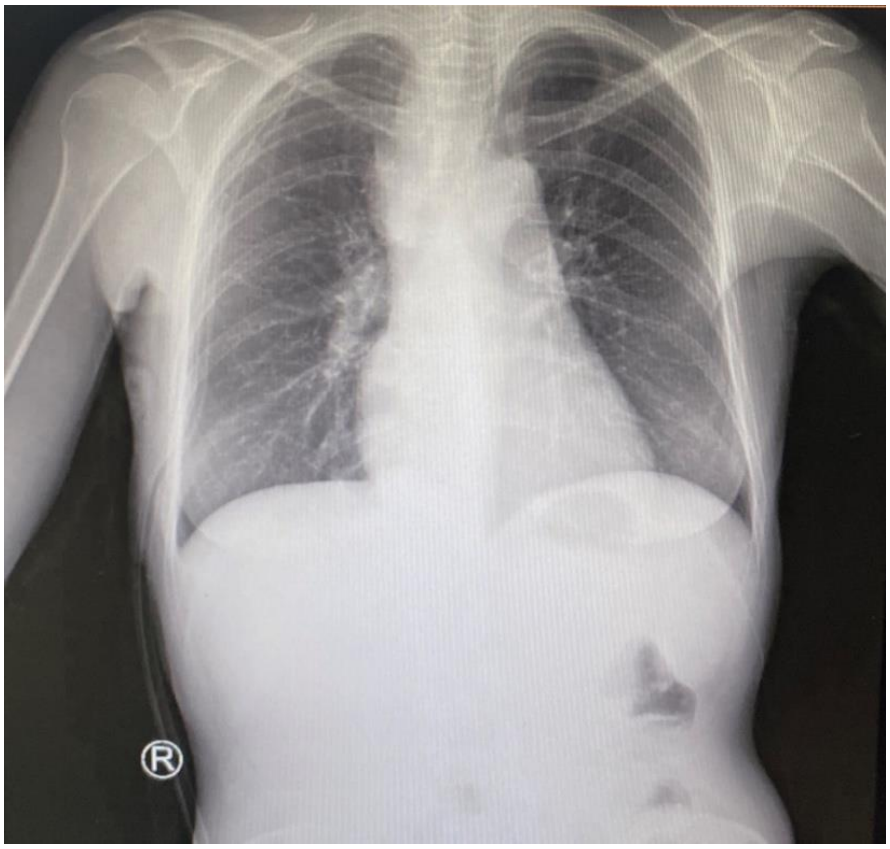

Figure 1. Chest X-ray showing infrahilar alveolar opacities and pleural effusion in moderate quantity on the right side

Taking into consideration the presentation particularities of the patient and the paraclinical findings, an aggressive disease was suspected, and the computed tomography (CT) +scan was decided. It showed a tumor in the right basal pleura with secondary pleural effusion of 40 mm (Figure 2), multiple mediastinal adenopathies bilateral and confluent, present in all lymph node groups, most of them necrotic, mediastinal and hilar adenopathies bilateral, gastric pericardial, lumboaortic, and possibly pelvic peritoneal adenopathies. Areas of ground glass opacities located in the lateral middle lobe and inferior right lobe at the Fowler level were also seen. In the left costodiaphragmatic recess, ground glass opacities were identified similar to those on the right side. In addition, pulmonary parenchymal changes were seen, more extensive on the right side with an uncertain substrate, and bilateral renal changes, more extensive on the right side with a post-pyelonephritic residual/secular type appearance (Figure 3). Moreover, a tumor of the right breast in the upper outer quadrant of approximately 7 mm and a tumor in the utero-vesical recess of a maximum of 7/10 mm were revealed (Figure 4).

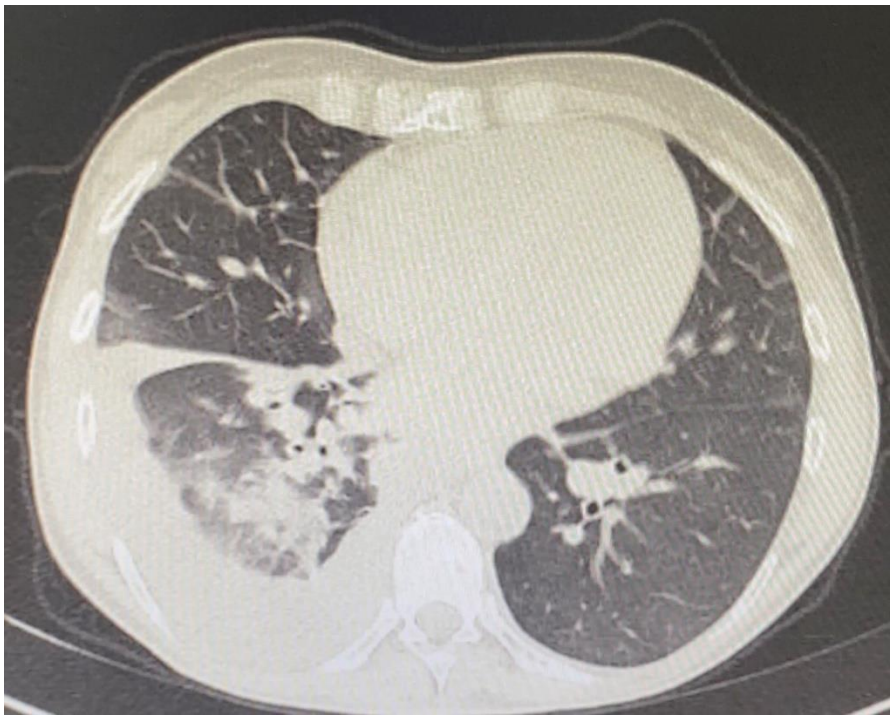

Figure 2. Chest CT scan showing a tumor in the right basal pleura with secondary pleural effusion of 40 mm

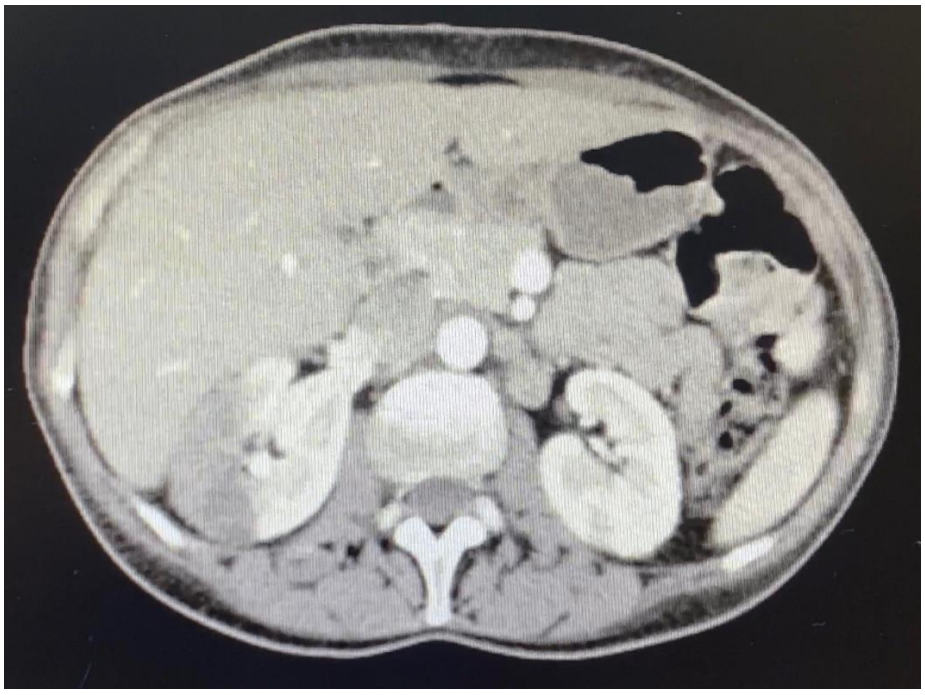

Figure 3. Abdominal CT scan showing bilateral renal changes, more extensive on the right side with a post pyelonephritic residual/secular type appearance

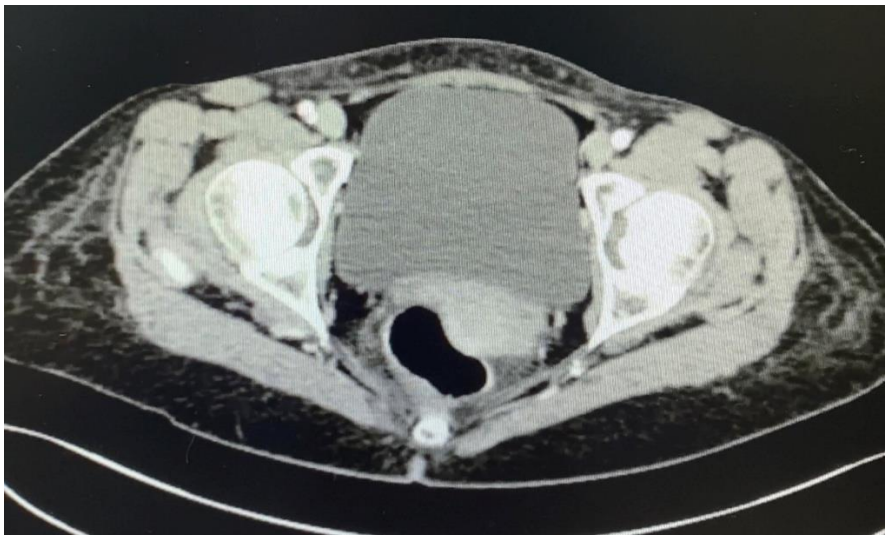

Figure 4. Pelvic CT scan showing a tumor in the utero-vesical recess of maximum 7/10 mm

The patient was seen by the gastroenterologist, general surgeon, otorhinolaryngologist and gynecologist. No signs of active bleeding were evident at the clinical examinations. Given the right lumbar pain and the post-pyelonephritic appearance of the right kidney, a urine culture was performed, showing no sign of a bacteria or yeast infection (negative). The upper gastrointestinal endoscopy did not detect any pathological processes in alignment with the current symptomatology. A Schatzki ring and a small trans-hiatal gastric hernia were identified. A colonoscopy cannot be performed entirely due to insufficient preparation. Sigmoidoscopy was performed, revealing uncomplicated hemorrhoidal disease.

Together with the thoracic surgeon, it was decided that the most accessible biopsy site remains the lung lesion. Before surgery, a transthoracic echocardiogram was performed, showing the left ventricle at the upper limit of normal, non-hypertrophic, with preserved systolic function, severe aortic insufficiency due to the presence of a mobile, hyperechoic mass attached to the aortic valve (tumor? vegetation?). Mild mitral insufficiency, mild pulmonary hypertension, pericardial fluid in a minimal amount (4 mm), dilated cavities, normal kinetics, left ventricle ejection fraction of 55% and diastolic function with delayed relaxation were also seen.

The transesophageal echocardiography confirmed the presence of a band (thrombus) on the aortic valve supposing that it was most likely a non-infectious endocarditis (marantic endocarditis). Mild to moderate aortic regurgitation was also seen.

Tumoral markers such as CA 125 >1000 U/mL, CA 15-3 154 U/mL, CA 19-9 149 U/mL and CEA 4.28 ng/ml were performed, noticing the highest increase among CA 125.

Multiple diagnoses were considered. Considering the location of the lesions identified on CT, it could have been a solid tumor - non-microcellular lung cancer, breast cancer, synchronous tumors, renal cell cancer, or uterine cancer - or a hematological malignant disease such as large B-cell malignant non-Hodgkin's lymphoma or peripheral T-cell lymphoma. The patient underwent pre-anesthetic and thoracic surgery consultations prior to the lung tumor biopsy. Thoracoscopy with mass biopsy of the right pleural tumor was conducted for histopathology and immunohistochemistry. Locally, a pleural drainage catheter was inserted.

Upon returning to the internal medicine department, the patient was bradylalic and bradypsychic, without motor deficits, nystagmus, or metabolic changes. An expansive intracranial process was suspected, and brain CT was performed, revealing a left-high parietal metastasis (5 mm) with small perilesional edema (Figure 5), that reduced in size (Figure 6) after intravenous corticosteroid treatment.

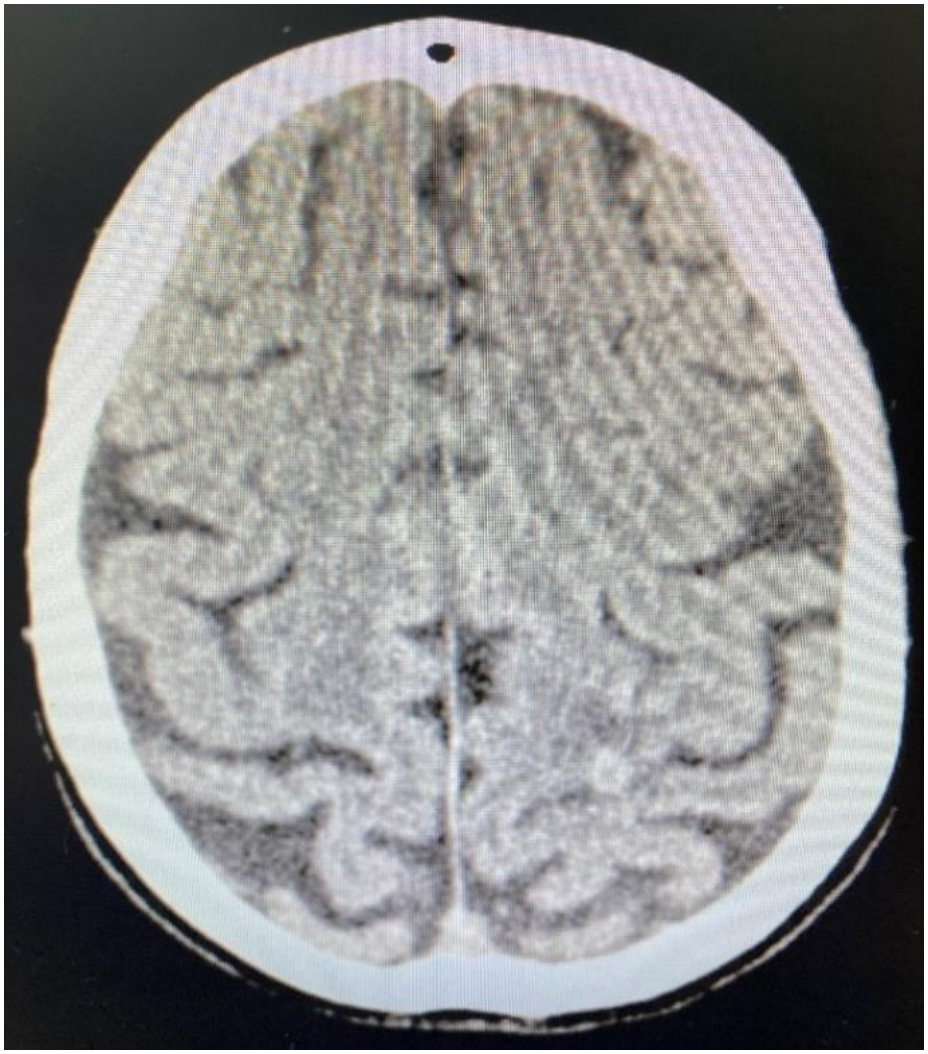

Figure 5. Brain CT revealing left-high parietal metastasis (5 mm) with small perilesional edema

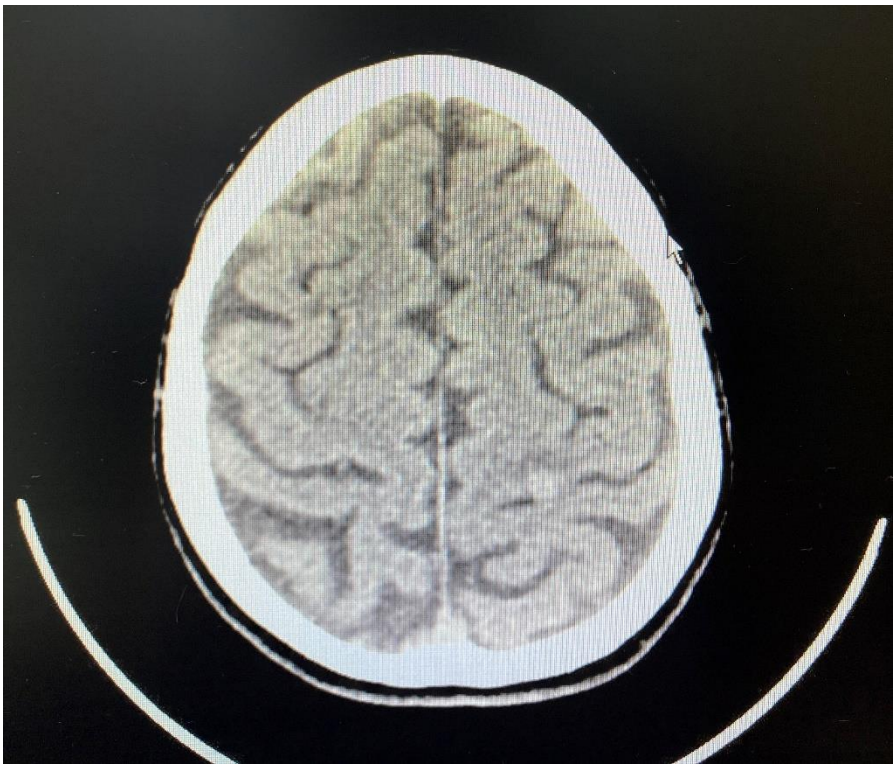

Figure 6. Brain CT revealing reduction in the size of the perilesional edema after intravenous corticosteroid treatment

During hospitalization the patient received loop diuretic (Furosemide 1 dose x3 daily), anticoagulant - prophylactic dose (Enoxaparin 0.4 ml daily), antihypertensive (ACE inhibitor - Enalapril 10 mg when systolic blood pressure exceeded 140 mmHg), nonsteroidal anti-inflammatory drugs if needed (Diclofenac), antiemetics if needed (Metoclopramide) and later she received corticosteroid treatment to reduce the perilesional edema of the left-high parietal metastasis (Dexamethasone once daily). Leukocytosis with neutrophilia, inflammatory syndrome (CRP 73.55 mg/L) and the multiple necrotic adenopathies identified on the CT scan examination made us initiate antibiotic treatment with piperacillin/tazobactam 1 dose every 8 hours (5 days), replaced after the transthoracic echocardiogram result with ceftriaxone 2g daily (8 days) to cover any bacterial pathogen involved in endocarditis, given the ambiguity of the etiopathogenity of the aortic valve mass (vegetation, tumor, thrombus).

The patient was discharged with moderate anemia (hemoglobin 10g/dL) and clinical sensory-motor peripheral neuropathy in the right arm. She was advised to stop smoking, to get an outpatient mammography and colonoscopy re-evaluation and to get a cardiology re-examination and a cardiac MRI to differentiate infective endocarditis from marantic endocarditis or aortic valve metastasis.

She was referred to an oncology clinic even though the tumor histopathological characterization and immunohistochemistry were not ready. In addition, she was advised to take iron tablets 2 capsules daily, before lunch and folic acid 5 mg daily for 3 months. Oral corticosteroids (32 mg daily) and pantoprazole (40 mg daily) were given for another 7 days to ameliorate the perilesional edema of the left-high parietal metastasis. Famotidine 40 mg daily was prescribed to prevent acid reflux given the Schatzki ring fragment and trans-hiatal gastric hernia. There were also prescribed metoclopramide if

nausea occurs, diclofenac 25 mg daily if needed (pain) and enalapril 10 mg at systolic blood pressure over 140 mmHg.

Histopathological examination from the pleural tumor biopsy had shown the appearance of a carcinoma with large epithelioid cells and clear and eosinophilic cytoplasm. Tumor cells were arranged in solid structures. An increased mitotic rate and necrosis were present. The histopathological specimen revealed a pleural metastasis of poorly differentiated large-cell carcinoma. The aspects pointed towards an adenocarcinoma of bronchopulmonary origin.

Immunohistochemistry was needed to differentiate a large B-cell malignant non-Hodgkin's lymphoma or a peripheral T-cell lymphoma from an adenocarcinoma given the fact that they are histologically similar. It was revealed by immunohistochemistry: CK-7 + and CK-20 - in tumor cells – pattern characterized of metastatic lung adenocarcinomas,[3] Napsin A + with moderate intensity in lung cells – expressed in over 80% of lung adenocarcinomas,[4] Nuclear PTR1 + with moderate intensity in tumor cells, CK34BE12 + membranous and focal cytoplasmic in tumor cells, GATA3 - in tumor cells, Ki67 nuclear index and in tumor cells = 10%. Immunohistochemistry revealed adenosquamous carcinoma of primitive pulmonary origin.

DISCUSSION

It was discussed the case of a young patient, without known pathology, who presented herself to the emergency room with severe symptomatic anemia, diagnosed with lung cancer, with probably multiple metastases in less common sites such as the breast, vesicouterine pouch and maybe the kidney, for which a biopsy was performed from the most accessible tumor process.

The non-metastatic extrapulmonary manifestations present in this case were metabolic (weight loss, fatigue), vascular, hematological (non-bacterial thrombotic endocarditis/marantic endocarditis, which occurs very rarely, severe microcytic hypochromic anemia, chronic disseminated intravascular coagulation) and neurological (sensory-motor peripheral neuropathy at right arm level).

While non-bacterial thrombotic endocarditis is a rare paraneoplastic finding, it is common in lung adenocarcinomas [5]. Marantic endocarditis is usually silent, as it is also seen in our patient presentation.

Disseminated intravascular coagulation (DIC) as a paraneoplastic phenomenon is common in non-small cell lung cancer patients, especially in advanced stages (III or IV). Usually, DIC appears in any solid tumor [6]. No active bleeding was identified in our patient, as it is less common in chronic DIC that develops over a longer period (weeks or months), but a hypercoagulability status (heavy smoker, malignancy, female gender) conducted to non-bacterial endocarditis [7].

A differential diagnosis was made between a solid tumor – adenocarcinoma (non-microcellular lung cancer, breast cancer, synchronous tumors, renal cell cancer, uterine cancer) and a hematological malignant disease – large B-cell malignant non-Hodgkin's lymphoma or peripheral T-cell lymphoma, given the location of the lesions identified on CT TAP and because from a histological point of view, they are similar to each other.

Having seen the histopathological and immunohistochemistry results, but also the aspect of the CT scan, we concluded that the patient had adenosquamous carcinoma in the right lung, with extensive lymph nodes involvement and with multiple metastases, placing the disease in cT2aN3M1c, stage IV B [8].

Molecular testing should be performed if adenocarcinoma is present in the biopsy specimen of an otherwise squamous tumor. Biomarkers such as EGFR mutations can be present in adenosquamous carcinoma [9,10].

Adenosquamous carcinoma is a rare type of non-small cell lung cancer that occurs in 0.4 - 4% of patients diagnosed with malignant

lung cancer. It is a mixed type, having components of both adenocarcinoma and squamous cell carcinoma. To be identified as adenosquamous, the tumor needs to have at least 10% of each cellular subtype. This type of lung cancer has a low incidence, increased aggressiveness, and poor prognosis rates. The overall survival rates in all stages are 53.5% at 3 years and 25.6% at 5 years [11].

There is no specific treatment for this type of cancer. Current treatment is based on guidelines for non-small cell lung cancer. Surgical excision (lobectomy with lymphadenectomy) is recommended in curative cases. For patients with EGFR+, tyrosine kinase inhibitors (erlotinib, gefitinib) represent the first-line treatment. The platinum doublet (4 cycles) is considered the first line in the case of EGFR-positive patients or if the molecular profile is not known [10,11].

At discharge, the patient presented a relatively good performance status (ECOG grade 2). She was referred to an oncologist to perform molecular testing (EGFR, ALK, KRAS, ROS1, PD-L1) before starting a targeted treatment.

CONCLUSIONS

We reported the diagnostic path of a rare subtype of lung cancer that is usually diagnosed after the surgical removal of the tumor, presented in a young female without known comorbidities. The patient had an atypical presentation with multiple extrapulmonary non-metastatic manifestations, of which the most important were marantic endocarditis (a very rare complication), severe microcytic hypochromic anemia, and chronic disseminated intravascular coagulation. A tissue biopsy was performed from the most accessible region: 1/3 inferior right thorax, posterior axillary line. Immunohistochemistry revealed adenosquamous carcinoma of primitive pulmonary origin, a cT2aN3M1c, stage IV B.

In this case, the tumor's particularity lies in the multiple metastases presented in less common sites (breast, vesicouterine pouch, and probably kidney).

Author Contributions:

A.D.S and R.M.V conceived the original draft preparation. A.D.S, C.C.D. and R.M.V were responsible for conception and design of the case report. A.D.S, C.C.D. and R.M.V was responsible for the data acquisition and for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review. All authors contributed to the critical revision of the manuscript for valuable intellectual content. All authors have read and agreed with the final version of the manuscript.

Compliance with Ethics

Requirements: *"The authors declare no conflict of interest regarding this article".*

Acknowledgments: *None.*

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Case Report

The risk-benefit balance of anticoagulant treatment: case report

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Abstract: *A 76-year-old female presents at the emergency department with dyspnea and pleuritic, sharp right laterothoracic pain that started suddenly. Her medical history includes stage 2 hypertension, diabetes mellitus type 2, chronic obstructive pulmonary disease stage IV GOLD with home oxygen therapy, chronic pulmonary heart disease, diffuse interstitial lung disease with a previous episode of alveolar hemorrhage, chronic renal disease stage 2, and paroxysmal atrial fibrillation, for which she had a Watchman device implanted, taking into consideration her anticoagulation contraindication due to the previous alveolar hemorrhage episode. The biological findings reveal hypoxemia and hypocapnia, a positive D-dimer test, an inflammatory syndrome, mild hypopotassemia, acute decompensation of chronic renal disease, and a positive urine culture with Enterococcus faecium. Emergency thoracic computed tomography reveals bilateral pulmonary thromboembolism. Immediate parenteral anticoagulation and antibiotic therapy are initiated with favorable evolution. At discharge, concerning the risk-benefit balance of anticoagulation in a senior patient with multiple comorbidities, the anticoagulant therapy is changed to a novel oral anticoagulant for at least three months, with reevaluation needed after that period.*

Keywords: *thromboembolism, warfarin, stroke, atrial fibrillation.*

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Introduction

Thrombosis is the formation of a blood clot that causes complete or partial blockage within an arterial or venous vessel, limiting normal blood flow. In developing countries, this pathology is associated with the three most common causes of death: myocardial infarction, stroke, and pulmonary embolism [1]. The mainstay of treatment for this spectrum of pathologies is anticoagulation.

Jay McLean discovered the first anticoagulant, unfractionated heparin, in 1916; it was the only substance used until 1940, when Warfarin, a compound that inhibited the synthesis of vitamin K-dependent coagulation factors II, VII, IX, and X, become available [2]. After 1980, new research on the coagulation cascade led to the development of novel oral anticoagulants that target the X factor [2].

Case Presentation

A 76-year-old female, an ex-smoker, presents at the emergency department with dyspnea and a sharp right laterothoracic pain that started suddenly. The patient was discharged from our hospital one week prior to the current presentation, when she was treated for urosepsis.

Her medical history includes stage 2 hypertension, diabetes mellitus type 2, chronic obstructive pulmonary disease stage IV GOLD with home oxygen therapy, chronic pulmonary heart disease, diffuse interstitial lung disease with a previous episode of alveolar hemorrhage, chronic renal disease stage 2 and paroxysmal atrial fibrillation. After the first episode of atrial fibrillation, which was over ten years ago, she had a catheter ablation of the arrhythmogenic foci, with a recurrence of the arrhythmia two years later. In order to prevent thromboembolic events, warfarin was added to her medication at that time; however, an episode of diffuse alveolar hemorrhage confounded the case, so the anticoagulant therapy was discontinued. Considering the relative contraindication to oral anticoagulation, a Watchman device was implanted to prevent embolic events by blocking the left atrial appendage.

Her at-home medications include a beta blocker, a statin, a diuretic, an antiplatelet agent, and a bronchodilator.

The clinical examination reveals deterioration of the patient's general condition, atrial fibrillation with a rapid ventricular response of 120 beats per minute, arterial hypotension with a blood pressure of 100/50 mmHg, symmetrical vesicular breath sounds without additional rales, oxygen saturation of 80% in breathing room air, corrected to only 91% with oxygen mask with a debit of 15 liters/minute, and no signs of deep venous thrombosis.

The clinical examination of the abdomen shows tenderness in the epigastrium and right hypochondrium upon superficial palpation.

The biological findings include a positive D-dimer test, an inflammatory syndrome, significant leukocytosis with neutrophilia, mild hypopotassemia, a glomerular filtration rate of 67 ml/min/1.73 m^2 (calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation) and normal amylase and lipase levels. The arterial blood gas analysis reveals the presence of hypoxemia and hypocapnia. The urine culture is positive for Enterococcus faecium.

The chest X-ray reveals a fine reticular pattern with left-sided pleural effusion and an increased cardiothoracic ratio (Fig. 1).

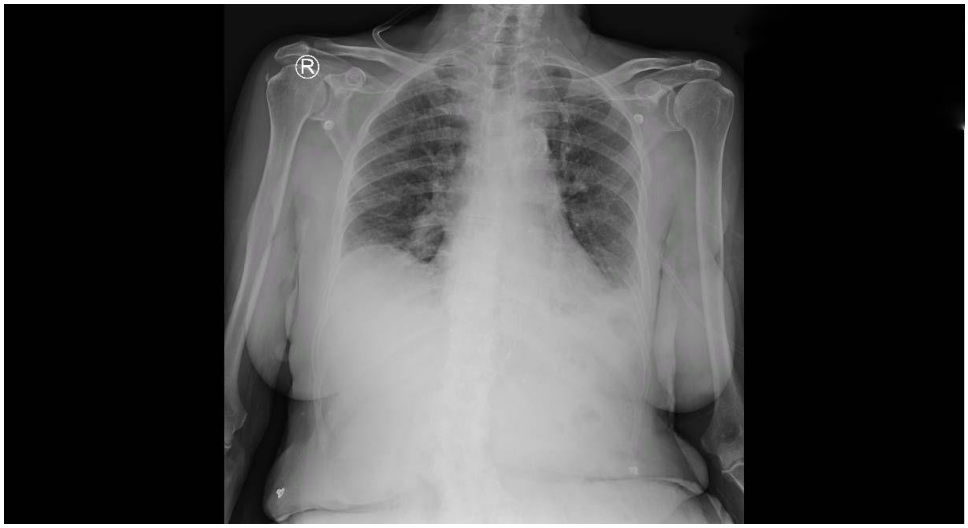

Fig. 1. Chest X-Ray image showing fine reticular pattern and left-sided pleural effusion

The electrocardiogram shows atrial fibrillation with a rapid ventricular response of 150 beats per minute, ST-segment elevation of 1 mm in aVR and diffuse ST depression.

The cardiac ultrasonography reveals a non-dilated left ventricle with concentric hypertrophy and preserved ejection fraction, a dilated right ventricle of 38 mm with tricuspid regurgitation jet gradient of 51 mmHg and dilated inferior vena cava. No pericardial effusion was present. Venous Dopper ultrasound confirms no signs of deep vein thrombosis. The abdominal ultrasonography is normal.

We continue the investigations with emergency thoracic computed tomography, which reveals a contrast agent filling defect in the terminal portion of both pulmonary arteries extending over the lobar ramifications. Other findings include bilateral pleurisy, a well-placed Watchman device, and aortic valve calcification (Fig. 2).

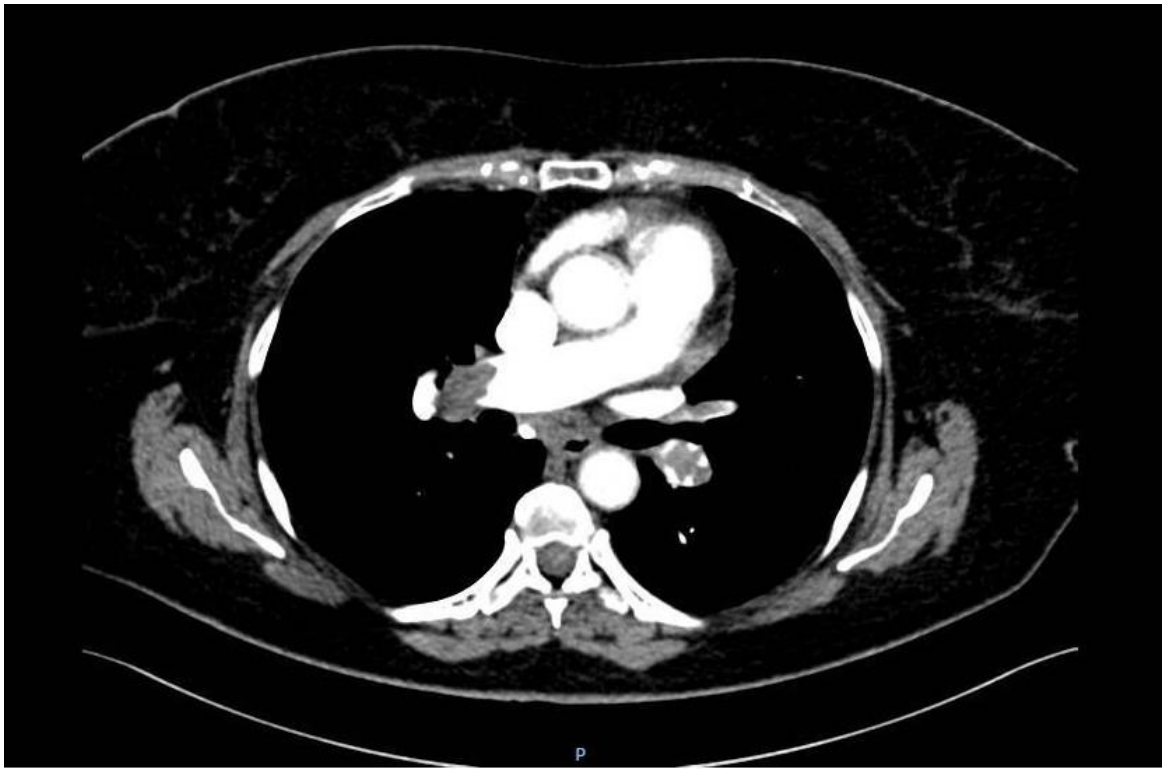

Fig. 2. Contrast-enhanced computed tomography image showing contrast agent filling defect in the terminal portion of both pulmonary arteries

The final diagnosis is acute respiratory failure due to bilateral pulmonary embolism and urinary tract infection. In addition to her previous doses of diuretics, angiotensin-converting enzymes, and bronchodilators, parenteral anticoagulant therapy and oxygen therapy were initiated, to maintain an oxygen saturation of 88-92%, antibiotic therapy, an increased dose of beta-blockers, and proton pump inhibitors. During the first few days of hospitalization, the patient develops severe hypotension, necessitating noradrenaline vasopressor support. Over the following days, the clinical and biological evolution is positive, the vasopressor support is discontinued, and the biological panel returns to normal. The oxygen requirement to maintain optimal saturation drops to 10 liters per minute.

At discharge, the parenteral anticoagulant is switched to a novel oral anticoagulant – Apixaban, in a low dose of 2.5 mg twice a day due to a creatinine level over 1,5 mg/dl and body weight of less than 60 kg and receives a recommendation for continuing aspirin, beta blockers, diuretics, angiotensin-converting enzyme inhibitors, statins, bronchodilators and home oxygen therapy. The oral anticoagulant is recommended for at least three months, with further need for reassessing the risk-benefit balance of anticoagulation in a senior patient with atrial fibrillation with a Watchman device implanted, acute pulmonary thromboembolism, and a history of diffuse interstitial lung disease and alveolar hemorrhage.

Discussion

The particularity of this case consists of the necessity of reassessing the risk-benefit balance of anticoagulation in a senior patient with multiple comorbidities.

While on anticoagulation therapy with warfarin in order to prevent thromboembolic events in atrial fibrillation, the patient presented diffuse alveolar hemorrhage which is an uncommon clinical condition that can be

caused by a coagulopathy induced by warfarin therapy. The narrow therapeutic window and multiple interactions with other medications might have been the cause [3].

Even though anticoagulation is the gold standard in preventing stroke and systemic embolization in atrial fibrillation, in cases of partial contraindication, as in our case with the alveolar hemorrhage, patients can benefit from the Watchman device, which closes the left atrial appendage and prevents further embolization. Studies show that this device is non-inferior to Warfarin in preventing embolic events [4]. Following implantation, anticoagulant management consists of Warfarin and Aspirin for 45 days, followed by dual antiplatelet therapy for six months, and then lifelong Aspirin [4].

As in our case, a new pathological event that might impact this management may be pulmonary thromboembolism. According to the 2019 Guidelines of the European Society of Cardiology on Acute Pulmonary Embolism, therapeutic anticoagulation for more than three months is recommended for all patients with pulmonary embolism as a Class I, Level A recommendation [5]. The significant, temporary, or reversible nature of risk factors influences the decision to continue oral anticoagulant medication after this period. Our patient had no significant risk factors, two moderate risk factors (respiratory failure and urinary tract infection one week prior to the occurrence), and three weak risk factors (aging, arterial hypertension, and limited mobility) as predisposing factors.

Venous thromboembolism, clinically presenting as deep vein thrombosis or pulmonary embolism, is globally the third most frequent acute cardiovascular syndrome after myocardial infarction and stroke [6]. A personal history of pulmonary embolism is one of the most significant risk factors for pulmonary embolism, as the risk is almost eight times greater in individuals aged 80 years than in the fifth decade of life [6]. Other recommendations to prevent a new episode include daily physical activity, which might also prevent muscular deconditioning in the elderly, weight management, avoiding infections and using compression stockings.

Conclusion

In conclusion, this case report emphasizes the importance of reassessing the risk-benefit balance of anticoagulation in a frail patient with multiple comorbidities. The efficacy of anticoagulation therapy in various pathologies remains debatable and is at the center of important decisions. Patient-centered medicine should lead us to provide the most individualized medical care, always considering the patient as an individual who requires a customized therapeutic approach.

Author Contributions:

A.T. conceived the original draft preparation. A.T. was responsible for conception and design of the review. A.T. was responsible for the data acquisition and for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review.

Compliance with Ethics Requirements: *"The author declares no conflict of interest regarding this article".*

Acknowledgments: *None.*

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Case Report
Hyponatremia in malignant neoplasia: A case report

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Abstract:
Hyponatremia is a frequent electrolyte imbalance with diverse etiology that may occur secondary to chemotherapy or autoimmune pathologies. We report the case of a 76-years-old female who presented for symptoms of severe hyponatremia like digestive intolerance, nausea, vomiting, generalized muscle weakness and vertigo, with a serum sodium level of 110 mEq/L. The patient was known with multiple cardiovascular and malignant comorbidities, being diagnosed with a right breast neoplasm with right radical mastectomy and axillary lymphadenectomy. Subsequently, multiple adenopathies were detected, which is why the patient followed several series of chemotherapy with trastuzumab and capecitabinum. These drugs can cause side effects, such as xerophthalmia, xerostomia, which are also frequently encountered in autoimmune pathologies. Hyponatremia is one of the most frequent side effects of capecitabinum. All these side reactions were investigated later, thus following the immunological tests, the diagnosis of Sjogren's syndrome was established. After ruling out other causes, it was established that hyponatremia appeared secondary to the recent administration of capecitabinum or within a syndrome of inappropriate antidiuretic hormone secretion (SIADH), which can be the result of a Sjogren's syndrome.

Keywords: *hyponatremia, Sjögren syndrome, malignant neoplasia*

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1. Introduction

Hyponatremia, defined as a serum sodium concentration less than 135 mmol/L, is one of the most frequent causes of hospitalization. This can appear as an epiphenomenon secondary to chemotherapy or in very rare cases it can appear within a syndrome of inappropriate antidiuretic hormone secretion (SIADH). SIADH can appear in Sjogren's syndrome, an autoimmune pathology, which can be triggered in a neoplastic context [1,2].

2. Case presentation

A 76-year-old-female presented for digestive intolerance, nausea, vomiting, generalized muscle weakness and vertigo, with onset 4 days before. The patient was known with multiple cardiovascular and neoplastic comorbidities. Ten years

previously (2011), she was diagnosed with right breast neoplasm with radical mastectomy and axillary lymphadenectomy, followed by 6 series of chemoradiotherapy. Three years later, she was diagnosed with thrombosis of the right axillary vein.

In 2016, during the clinical examination, a right laterocervical adenopathy was detected, which was biopsied, and after the histopathological examination, it turned out to be a metastasis of a poorly differentiated breast carcinoma. At that time, chemotherapy with capecitabine and trastuzumab was performed. However, the patient developed side effects, with the appearance of a left bundle branch block, accompanied by cardiac dysfunction, secondary to the administration of trastuzumab. Therefore, trastuzumab was replaced by lepatinib (a dual tyrosine kinase inhibitor that interrupts the HER2/neu and epidermal growth factor receptor pathways). In the same year, the patient underwent surgery for a herniated disc located at the level of the L5-L6 vertebrae.

In the following year, secondary to chemotherapy, the patient was diagnosed with ischemic heart disease, for which angioplasty was performed with an active pharmacological stent on the right coronary artery and circumflex artery. At the same time, the patient was evaluated by computed tomography (CT), where no oncological lesions were detected, so the treatment with capecitabine was continued.

In 2018, the positron emission computed tomography (PET-CT) scan highlighted metabolically active secondary left axillary, latero-cervical and retropectoral lymph node determinations and the treatment with capecitabine was continued.

Nevertheless, in 2019, an important regression of the previously described secondary determinations was observed at CT, but with multiple adenopathies located at the upper jugular level (14/12 mm), submental (9/6 mm), right submandibular (13.5/6 mm) and supraclavicular (9 mm). After cardiological examination, echocardiography revealed a left ventricular ejection fraction of 50%, which is why trastuzumab treatment was restarted plus carboplatin. In 2020, the patient stopped the chemotherapy on her own initiative.

A year later, the oncological evaluation revealed an axillary adenopathy <1 cm and a right laterocervical adenopathy of approximately 3 cm, hard, mobile, but with a tendency to fixate on the deep and superficial planes. CT scan revealed multiple mediastinal adenopathies, with maximum axial diameters of 28/18 mm at the left parathyroid level, increasing in size, several right supraclavicular adenopathies increasing in size 11.5/8 mm, but without images suspicious for secondary pulmonary, hepatic or bone lesions.

To initiate chemotherapy, the patient had again a cardiological examination. Echocardiography revealed a preserved ejection fraction of the left ventricle, so trastuzumab was administered at an interval of 21 days. One month later, in December 2021, the patient presented a rash consisting of violet, non-pruritic papules on the upper and lower limbs, which is why she was examined in the dermatology service, receiving a recommendation for oral and topical corticosteroids, with a slowly favorable evolution.

In December 2022, the patient stopped again any chemotherapy. However, the rash spread to the anterior and posterior chest, as well as the sacrum, with severe skin xerosis, as well as xerophthalmia and xerostomia, symptoms that have worsened recently. Therefore, the patient was admitted in a clinical hospital for infectious and tropical diseases, where a differential diagnosis between a cutaneous vasculitis in a Sjogren's syndrome, systemic lupus erythematosus or an undifferentiated collagenosis was discussed. Later, to confirm the diagnosis, extended Antinuclear Antibody (ANA) profile, total complement, C3 and C4 were recommended. The extended ANA panel revealed intensely positive SS-A, SS-B, Ro-

52 and U1-nRNP antibodies, so the diagnosis of Sjogren's syndrome was established.

Two months later, the patient was hospitalized for algoparesthetic lumbosciatica due to dyscartosis and marked asthenia. At the time, no metastases were visible on the CT scan, but only a few infracentimeter lymph nodes at the right subclavicular, paratracheal, left parotid level, without pleuro-pericardial effusion and the presence of dyscarthrosis at the level of the L2-L3 and L5-S1 vertebrae was detected. A cervical magnetic resonance imaging (MRI) was also performed, which highlighted bilateral parotid adenopathy, more pronounced on the right side, of approximately 14.8 mm. The suspicion of paraneoplastic collagenosis was raised and Medrol 16 mg, 1/4 cp/day and Plaquenil 200 mg, 1 cp/day were recommended, as well as an oncological re-evaluation, considering the patient's history.

One month later, the patient presented at the territorial oncology service, to reevaluate and restart chemotherapy. Thus, an Eastern Cooperative Oncology Group (ECOG) performance status of 1 was identified, with restrictions in performing demanding physical activities. Also, considering the progression of tumor markers (CA-125, CA-15.3 and CA-19.9), it was recommended to perform a PET-CT investigation, which revealed 2 nodular images, with increased metabolic activity, with maximum dimensions of 18/15.5 mm, considered to be either a Whartin tumor or thyroid adenopathies. Also, supradiaphragmatic adenopathies were identified, located at submental level (8.5/8 mm), left middle jugulo-carotid (11.5/8.5 mm), left supraclavicular (12/8 mm) and left paratracheal (maximum 15/13 mm).

At the time of admission in our internal medicine clinic, the patient presented an altered general condition. During the clinical examination, diffuse vasculitis-type skin lesions were observed on the anterior chest, right post-mastectomy scar and lymphedema of the right upper limb. Also, the patient had bilateral eyelid edema and palpation revealed adenopathies at the submandibular and right retrosternocleidomastoid level, apparently adherent to the deep planes. The patient was hemodynamically balanced but had a painful abdomen on palpation in the epigastrium and right hypochondrium.

At admission, the blood tests revealed a moderate normochromic, normocytic anemia (Hb 9.5 g/dL), severe hyponatremia (Na 110 mmol/L), a nitrogen retention syndrome (urea 77 mg/dL, creatinine 1.58 mg/dL), D-dimers with increased values (999 ng/mL), a mild hepatic cytolysis, as well as metabolic acidosis (pH 7.29, HCO3 17.3 mEq/L). The electrocardiogram revealed a known left bundle branch block aspect, secondary to administration of trastuzumab.

The routine chest X-ray showed a moderate pleural effusion on the left basal side and mild interstitial changes on the basal right side, as well as the presence of the chemotherapy chamber with the distal end projected at the level of the superior vena cava (Figure 1).

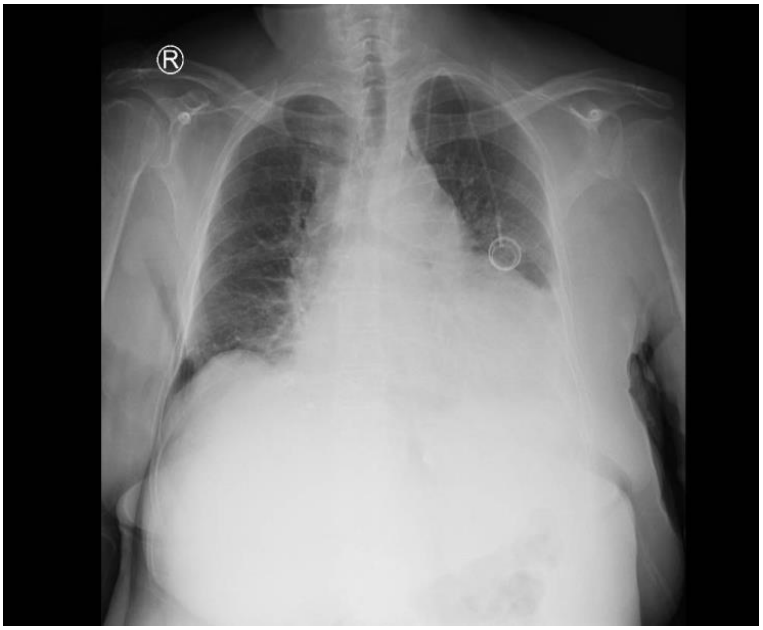

Figure 1. Chest X-ray showed a moderate pleural effusion on the left basal side.

During hospitalization, the patient underwent antihypertensive treatment with beta-blocker and calcium channel blocker, antiplatelet agent, anticoagulant in prophylactic dose, loop diuretic and thiazide, as well as hydro electrolyte rebalancing.

However, shortly after admission, the patient's condition deteriorated, complaining of persistent resting dyspnea, headache, and vertigo, considered to be secondary to severe hyponatremia. Sodium chloride was initiated on continuous infusion, as well as oxygen therapy, with a flow rate of 4 L/min.

An echocardiography was performed, which revealed concentric pericardial fluid of 30 mm and bilateral pleural effusion, 3 cm on the left and 4 cm on the right. Cardiovascular surgery and thoracic surgery consultation was requested. Cervico-thoracic CT scan with contrast substance was performed and reevaluation in view of thoracoscopic puncture and pleurisy.

At the same time, because the bilateral eyelid edema persisted, an ophthalmology consultation was requested, that established the diagnosis of upper eyelid stye in the right eye and nodular blepharoconjunctivitis in both eyes.

During admission, the patient received transfusions with erythrocyte mass, given a hemoglobin value of 7.42 g/dl, in the neoplastic context.

A control chest X-ray was performed, which revealed an important decrease in the size of the pleuro-pericardial fluid (Figure 2).

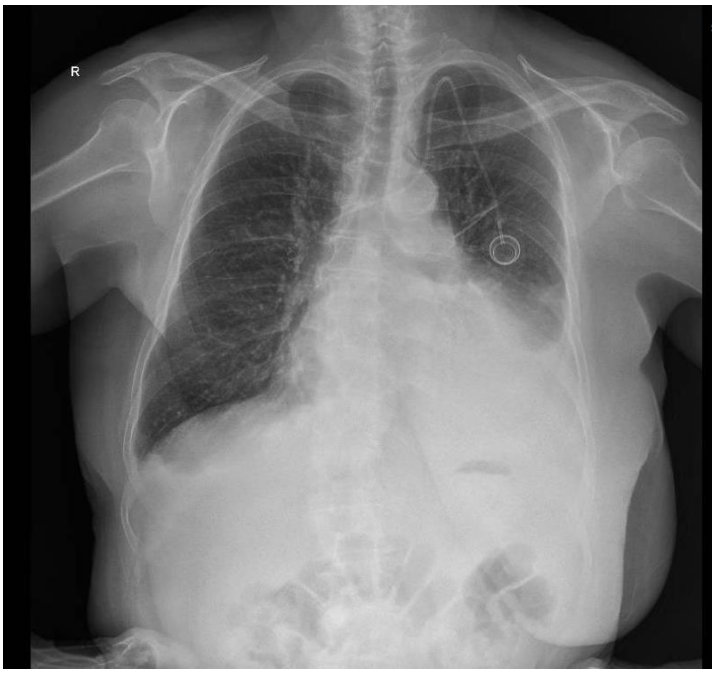

Figure 2. Control chest X-ray

Due to the favorable evolution, with decreasing pericardial and pleural effusion, the patient was treated only with diuretics. Under diuretic treatment, the symptoms ameliorated, with normalization of the serum sodium value. The patient was discharged a few days after, being hemodynamically and respiratory stable, without the need of oxygen therapy.

3. Discussion

We presented the case of a patient with an operated breast cancer, diagnosed with Sjogren's syndrome after chemotherapy, hospitalized with severe hyponatremia, which may be secondary to Sjogren's syndrome or chemotherapy.

Considering that the patient was previously evaluated oncologically with resumption of chemotherapy, her hyponatremia was considered to be due to the administration of capecitabine. Hyponatremia is one of the most frequent side effects of this drug, along with xerophthalmia, xeroderma, erythematous rash and arthralgia[3].

Also, from the patient's history, she was also treated with trastuzumab, a chemotherapeutic drug which, has as very frequent side reactions conjunctivitis, dyspnea, cough, diarrhea, vomiting, nausea, lip edema, abdominal pain, dyspepsia, constipation, erythema, transient rash. As side effects with a lower frequency, we mention xerophthalmia, papillary edema, pericardial exudate, pleural effusion, respiratory failure, xerostomia, xerophthalmia[4].

All these side reactions, which are also found in autoimmune diseases, were investigated later, thus following the performance of the extended ANA panel, the diagnosis of Sjogren's syndrome was established.

Sjogren's syndrome is a systemic autoimmune disease, which causes chronic inflammation of the exocrine glands, frequently affecting the lacrimal and salivary glands, but also the nose, upper respiratory tract, oropharynx and, in the case of women, even the vagina. This pathology has a female predominance, with a 10/1 women/men ratio and is one of the most frequent autoimmune diseases affecting middle-aged individuals[5,6].

Two types of Sjogren's syndrome have been described, primary and secondary. Secondary Sjogren's syndrome is associated with collagen diseases, such as rheumatoid arthritis, systemic lupus erythematosus,

systemic sclerosis, scleroderma and dermatopolymyositis[5,7].

The clinical characteristics of Sjogren's syndrome can be classified into glandular manifestations, which are related to exocrine dysfunction, and extraglandular manifestations, which affect other organs than the exocrine gland. Among the glandular manifestations, Sicca keratoconjunctivitis is described, which manifests with dryness, burning, photophobia, at the level of the salivary glands, symptoms such as xerostomia, dysphagia; loss of the sense of taste due to atrophy of the papillae may appear, but also other glandular effects, such as decreased bronchial secretions, hypochlorhidia, hypopepsinogenemia (atrophic gastritis) and rarely it can also affect the pancreas causing autoimmune pancreatitis[5–7].

The extraglandular manifestations are diverse and involve musculoskeletal system through arthralgias, arthritis, diffuse myalgia, skin damage with xerosis, erythemato-papular eruptions, Raynaud's syndrome, lung damage through interstitial fibrotic lesions and there may even be neurological damage causing sensitive peripheral neuropathy, mixed, mononeuritis multiplex, vegetative neuropathy. This pathology presents a risk of malignant transformation, potentially causing non-Hodgkin's lymphoma[5–7].

One of the easiest methods to quickly establish the diagnosis of Sjogren's syndrome is the complete immunological evaluation of a patient who presents xerophthalmia and xerostomia. The definite establishment of this diagnosis is made according to the American-European Consensus Group (AECG) criteria. These include:

1. Ocular symptoms - dry eyes for more than 3 months;
2. Oral symptoms - feeling of dry mouth for more than 3 months, recurrently swollen salivary glands;
3. Ocular signs - Schirmer test (< 5 mm in 5 min), positive vital dye staining results;
4. Oral signs - abnormal salivary scintigraphy findings, abnormal parotid sialography findings, abnormal sialometry findings (unstimulated salivary flow < 1.5 mL in 15 min);
5. Positive minor salivary gland biopsy findings;
6. Positive anti–SSA or anti–SSB antibody results [6,8].

The diagnosis of primary Sjogren syndrome requires at least four of these criteria. In addition, either criterion number 5 or criterion number 6 must be included[8].

4. Conclusions

There are very rare cases in whom Sjogren's syndrome is diagnosed after chemotherapy because the symptoms can be misleading, being considered in the context of the medication. Also, hyponatremia can appear within a SIADH, which can be secondary to Sjogren's syndrome or chemotherapy.

It has been demonstrated that the administration of capecitabinum causes a decrease in serum sodium levels, so in the case of patients with neoplasms, once chemotherapy with this drug has been initiated, the ionogram must be monitored.

Author Contributions:

D.G, A.D. and C.D. conceived the original draft preparation. D.G, A.D. and C.D. were responsible for conception and design of the review. D.G, A.D. and C.D. were responsible for the data acquisition and for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review. All authors contributed to the critical revision of the manuscript for valuable intellectual content. All authors have read and agreed with the final version of the manuscript.

Compliance with Ethics Requirements: *"The authors declare no conflict of interest regarding this article".*

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