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Editorial Board

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A new era for the Hellenic Journal of Surgery

Nikolaos I. Nikiteas

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The Hellenic Journal of Surgery (HJS) is the official scientific journal of the Hellenic Surgical Society, whilst its publication is considered the most important scientific activity of the society. The first issue was published in 1956, as a continuation of the "Bulletin of the Surgical Society", first published in 1947. The journal, which is now published in English, publishes papers from Greece and abroad. Since 2020, by decision of the Board of Directors, the journal is published in electronic format. Following a small break from its publication mainly as a result of the COVID-19 pandemic and a renewal of its editorial team,the journal is resuming its publication in 2023.

Given the opportunity, we would like to express our gratitude to the former editor-in-chief Dr. Nomikos Iakovos for his outstanding leadership and invaluable contribution to our medical surgery journal. His tireless dedication and unwavering commitment to excellence have undoubtedly helped maintain the high quality of the journal. His insightful guidance and rigorous editorial standards have not only upheld the integrity of the journal but have also inspired and motivated countless researchers and clinicians to strive for excellence in their work. We would like to thank Dr. Nomikos for his exceptional service and for setting the standard of excellence that we will continue to uphold.

Our journal will publish high-quality original papers in our field, state-of-the art reviews and meta-analyses, illustrative case reports, pictures/videos, "how I do it" articles, surgical history articles, brief communications, perspectives and editorials by experts in relevant fields of surgery. Developing fields such as robotic surgery, minimally invasive surgery, interventional radiology and surgical endoscopy will be strongly supported. Equally, articles focusing in basic and translational research related to surgery are welcome. Our journal aims to provide a rapid, yet high-quality review procedure, whereas the editors will actively contribute by curating special issues focused on topics of high interest.

The HJS welcomes authors from a wide spectrum of surgical subspecialties from all over the world. Apart from surgical specialties, authors with surgery-related specialties, such as interventional radiologists, gastroenterologists and medical oncologists, will be encouraged to contribute. We believe our journal will enhance dissemination of new ideas and expedite the application of new advances. Attracting young surgeons is major goal, while we hope our journal will stimulate interest and research is all fields of surgery. Moreover, we foresee to attract a broader readership that includes medical students, residents, physicians and surgeons in private practice, as well as those associated with government and private as well as academic institutions.

Our journal will strive to uphold the highest standards of scientific integrity and ethical conduct, ensuring that published research is rigorous, accurate, and transparent. Alongside the Co-Editors, an Advisory and an Editorial Board comprisedof distinguished colleagues from Greece and abroad will help our journal excel. We anticipate that HJS will surpass national boundaries and bring together not only surgeons but also other scientists from across the world. We promise to embody a "living" journal that will continue to change and evolve over time. While we embark on this new mission we welcome all young and senior surgeons to join us with the ultimate goal of making the HJS a successful, high quality journal.

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Original Article

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Appendiceal tumours as incidental findings in patients undergoing emergency appendicectomy: A retrospective, single-center study and a brief overview of current practice standards

Francesk Mulita[1], Levan Tchabashvili[1], Konstantinos Tasios[1], Andreas Antzoulas[1], Angelos Samaras[1], Elias Liolis[2], Ioannis Maroulis[1], Konstantinos Bouchagier[1], Georgios-Ioannis Verras[1]

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ABSTRACT

Introduction: Primary cancers of the appendix are very rare and most of them are usually found accidentally on appendectomies performed for appendicitis. Although these tumours are rare, there is a diverse histology.

Methods: We conducted a single-center retrospective study of patients undergoing appendectomy at our institution for the suspended diagnosis of appendicitis. From January 2003 to December 2018 a total of 1809 patients underwent appendectomy under general anaesthesia. Patient demographics, type of procedure, and tumour histology were recorded.

Results: The mean age of patients was 32 years (range, 14 to 85). Of these patients, 821 (45.38%) were female, and 988 (54.62%) were male. In total 959 (53.01%) underwent laparoscopic appendectomy and 850 (46.99%) underwent open appendectomy. An appendiceal neoplasm was found in 17 patients (0.94%). Of these 17 patients, four (23.53%) were reported to have benign tumours, while 13 (76.47%) were reported to have malignancies. The most frequent appendiceal tumour was carcinoid, which was detected in 10 patients (58.82%).

Conclusion: Tumours of the appendix are very rare and the majority of them are malignancies. Early recognition is very important. There is no standard of care due to the rare frequency of these tumours.

Key Words: *Appendiceal tumours; appendicitis; laparoscopic appendectomy; open appendectomy*

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INTRODUCTION

Primary appendiceal tumours consist of rare entities that occur in less than 2% of all performed appendicectomies, irrespective of preoperative diagnosis, and in about 0.7% to 1.7% of appendiceal specimens retrieved from patients with a preoperative diagnosis of acute appendicitis [1–3]. Pathological classification of appendiceal tumours has seen multiple revisions and re-classifications. The current practice consensus is focused around the revised

WHO 2019 classification, as well as the consensus statement issued by the Peritoneal Surface Oncology Group International (PSOGI) Executive Committee regarding mucinous appendiceal neoplasms and pseudomyxoma peritonei [1,4–8].

The small patient number in reported cohorts of appendiceal tumour patients, as well as the lack of RCTs largely owed to the incidental nature of this condition, has been a hurdle in the development of guidelines for their detection, management, and follow-up. The presence of diverse histological subtypes with differentiating predictive characteristics has further complicated the process. Appendiceal tumours can be broadly split into epithelial and neuroendocrine neoplasms [1–3,8]. Mucinous appendiceal tumours form a distinct subtype of the former, which is most commonly described using the PSOGI classification as the golden standard for pathological classification. The 2019 WHO classification, splits epithelial tumours of the appendix into the following categories: Hyperplastic polyp, Sessile serrated lesion with or without dysplasia, low-grade appendiceal mucinous neoplasm (LAMN), high grade appendiceal mucinous neoplasm (HAMN), adenocarcinoma, undifferentiated carcinoma, Goblet cell adenocarcinoma and neuroendocrine tumours (well- or poorly differentiated). A study reported a cumulative five-year survival rate of 83% for patients with any type of appendiceal tumour, indicating acceptable survival rates as a whole, but with many variations regarding different subtypes. Due to the scarcity and heterogeneity of reports on appendiceal tumours, estimates of the incidence rates of histological subtypes are also difficult to obtain [4–7,9]. The most common subtype of appendiceal tumours are neuroendocrine appendiceal neoplasms, with an incidence rate ranging from 35% to 85% of all appendiceal tumours (as identified by the largest patient series available through the SEER database), while other studies mention that adenocarcinoma of the appendix makes up for almost 60% of appendiceal tumours [4,9]. A recent study showed that patients that underwent interval appendicectomy after a case of appendicitis that was managed nonoperatively, had a pooled prevalence of 11% for appendiceal malignancies.

The primary clinical manifestation of appendiceal tumours is often non-specific and can vary between patients. Chronic distention of the appendix due to mucin accumulation might cause non-specific right lower quadrant pain, although this can also often be a late finding of advanced disease. Weight loss and chronic iron deficiency anaemia are also symptoms associated with appendiceal tumours. The most often acute presentation of appendiceal tumours is invariably acute appendicitis, caused by luminal obstruction evident by RLQ abdominal pain, elevation in inflammatory markers, anorexia etc. [4,9–16].

Although a number of retrospective reviews have presented several patient cohorts, there is still a scarcity of data regarding appendiceal tumours and their histopathological subtypes. This retrospective, single-center cohort study presents data on patients that were taken to the operating theatre with a diagnosis of acute appendicitis, underwent emergency appendicectomy and appendiceal tumours were found as the final diagnosis.

METHODS

We conducted a systematic, retrospective search of our institution's database for patients that underwent emergency laparoscopic or open appendicectomy due to acute appendicitis over a period of 16 years. Patient inclusion criteria included the following: 1) Definitive sonographic or CT-confirmed diagnosis of acute appendicitis preoperatively, 2) Presence of a finalised pathological report for the extracted specimen, 3) Patients that underwent emergency appendicectomy due to acute appendicitis either with a laparoscopic or open approach and 4) Patients that underwent surgery from January 1st 2003 to December 31st 2018. Patients with no definitive pathological report, patients that underwent interval appendicectomy, patients undergoing palliative surgery for known malignancies of the appendix (e.g. advanced stage pseudomyxoma peritonei with complications) and patients with a preoperative diagnosis of appendiceal neoplasm were excluded from the study.

After identifying qualifying patients for inclusion in the study, we tabulated the demographics of those that were ultimately diagnosed with appendiceal tumours vs patients without further appendiceal pathology. Statistical processing of the data utilised the Student's t-test for continuous variables and chi-squared two-sided tests for comparing proportions.

RESULTS

In total, 1809 patients that underwent emergency appendicectomy during the specified time period were identified. The mean patient age was 32.6 years (range 15 – 85). Of these patients, 821 (45.38%) were female and 688 (54.6%) were male. In total, 959 patients (53.01%) underwent laparoscopic appendicectomy and 850 (45.99%) underwent open appendicectomy. Appendiceal neoplasms were found intraoperatively in 17 patients in total, 0.94% of the total patients. Four of the patients (23.53% of the appendiceal tumour patients) were found to have benign

appendiceal tumours (adenomas and polyps). Of the remaining 13 patients with appendiceal malignancies, ten patients (58.82%) were diagnosed with appendiceal carcinoid tumours. Of the remaining patients, there were two patients with high-grade appendiceal mucinous neoplasms and one patient with low-grade appendiceal mucinous neoplasm (Table 1).

A total of 415 cases presented with complicated appendicitis, as opposed to 1394 cases of uncomplicated appendicitis. There was no statistically significant difference in the presence of complicated vs uncomplicated appendicitis in patients presenting with appendiceal neoplasms. Of the patients presenting with carcinoid tumours of the appendix, six (60%) presented with localised disease, three patients (30%) with locally advanced disease, and one patient with distant metastasis evident in postoperative staging. Tumour size was less than 1cm in six patients (46.1%), between 1 and 2 cm in three patients (23.07%), between 2 and 5 cm in two patients (15.3%), and more than 5cm in two patients (15.3%) (Table 2). The sum of the patients experienced uneventful postoperative periods and were discharged home without further complications. In seven patients (53.8%), the operation was converted to right-sided hemicolectomy either due to the size of the primary tumour, or the presence of locally advanced disease. Average hospital stay differed between patients diagnosed with appendiceal tumours, vs patients that had either complicated or uncomplicated appendicitis without malignancy (5.7 days vs 3.26 days, $p < 0.05$). Age, gender, and rates of postoperative complications did not differ significantly between the two subgroups.

TABLE 1: Descriptives of appendicectomy patients.

Descriptives	No of Patients
Age	32.6 (15-85)
Gender	
Female	821 (45.38%)
Male	688 (54.6%)
Operative Approach	
Laparoscopic	959 (53.01%)
Open	850 (45.99%)
Appendiceal Tumours (Total)	17 (0.94%)
Benign Appendiceal Lesions	4 (23.53%)
Appendiceal Carcinoid	10 (58.82%)
HAMN	2 (11.7%)
LAMN	1 (5.85%)

TABLE 2: Characteristics of appendiceal tumour patients.

Characteristic	No of Patients
Diameter Size (cm)	
<1	6 (46.1%)
1-2	3 (23.07)
2-5	2 (15.3%)
>5	2 (15.3%)
Conversion rate	7 (53.8%)
Average Hospital Stay (days)	5.7
Disease Stage	
Local	6 (46.1%)
Locally Advanced	3 (23%)
Distal Metastasis	1 (0.05%)

DISCUSSION

The first-ever report of an appendiceal mass attributed to a tumour was published in 1882, with the first case series being published in 1903 [12,13]. Since then, appendiceal tumours remain a largely elusive ailment, with physicians struggling to produce large reports of patient series that would bring about the creation of definitive management guidelines. To complicate matters further, histological subtyping of appendiceal tumours reveals a large number of distinct histological subtypes, often associated with differences in patient prognosis. Perhaps the greatest issue regarding appendiceal neoplasms is that they more often than not become symptomatic at advanced stages, or present as a bout of acute appendicitis, meaning that they leave little room for early diagnosis and management, especially in an elective fashion.

Our results seem to be in line with previous findings of the largest studies on appendiceal tumours. With an incidence rate of less than 2% overall in patients that are incidentally found to be ailed by appendiceal tumours and a small prevalence of males over female patients, our experience further confirms current findings [10–13,17-20]. Appendiceal tumours are usually located at the tip of the appendix, with a diameter of less than 1cm in most cases. Although this was also true in our patient cohort, we did encounter patients with neoplasms larger than 1cm (53.9%). This difference can be attributed to the selective inclusion of patients undergoing emergency appendicectomy with a preoperative diagnosis of acute appendicitis alone, rather than the inclusion of both emergency and elective surgery of neoplasms that is expected to apply to earlier-stage carcinomas [10].

Perhaps the most controversial aspect of appendiceal

malignancies is histological classification. The 2019 update in the WHO classification of appendiceal tumours, brought about several changes that need to be discussed and kept in mind by surgeons treating appendiceal tumours. The term "sessile polyp" was replaced by "sessile lesion", now indicating that a polypoid formation is not necessary to diagnose a sessile lesion of the appendix, a change that could lead to a rise in the incidence of appendiceal neoplasm diagnosis [10,11]. The WHO 2019 classification, also moved closer to the PSOGI classification of mucinous neoplasms of the appendix, which abandoned complex nomenclature, often found to have little to no effect in the effective clinical classification of patients and patient survival outcomes. Tumour grade is now considered the cardinal characteristic, with LAMNs being included as Grade I neoplasms, and HAMNs being Grade 2 (primarily) and Grade 3 tumours [10,11]. It is essential that the surgeon assigned to such cases is familiar with the new nomenclature since it closely correlates with patient management strategies and the referral to the oncologist.

In a 2021 statement, a joint force of the PSOGI and EURACAN committees published what are now the latest clinical-oriented management guidelines for appendiceal tumours based on the latest classification changes. Inclusion of preoperative CEA and CA 19.9 in preoperative evaluation of patients is now strongly recommended after several studies proved that the levels of these biomarkers do not only correlate with the presence of appendiceal malignancy, but also with the survival rates of patients [17-23]. Due to the nature of our study cohort (emergency surgical patients) we did not have the ability to evaluate such biomarkers. Simple polyps that do not exhibit malignant cells can be managed by appendicectomy alone, as was done in our patients as well. Carcinoid tumours of the appendix that consisted the largest subgroup of malignancy patients, require a therapeutic right-sided hemicolectomy if they are larger than 1-2 cm in diameter, mesoappendiceal invasion of more than 3 mm or with high rates of Ki67 indexes [21,22, 24-26]. Macroscopic evidence of peritoneal spread is now considered an indication for either the use of intraoperative HIPEC, or adjuvant chemotherapy. One area of remaining debate is the management of LAMN and HAMN occurrences for which no clear recommendations can be made. Although authors have described the expectant management strategy for LAMN lesions, recurrence rates seem to indicate the need for more radical approaches. As of now, despite the low level of evidence, HAMN lesions are managed aggressively, in a similar fashion to adenocarcinoma. These gaps in knowledge have been the reason for a more radical approach after our intraoperative diagnosis of appendiceal neoplasms, with right-sided hemicolectomies being performed in most patients with gross disease irrespective of histological subtype [24–28].

CONCLUSIONS

Appendiceal tumours are an extremely rare, mostly incidental finding. Despite their rarity, their biological behaviour can vary according to the histological subtype and can manifest as aggressive malignancy. Lack of patient data is the main reason behind gaps in current knowledge regarding their management. Our work presents the 15-year experience of a tertiary center in the management of appendiceal malignancies.

Conflict of interest: *There are no conflicts of interest to declare.*

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Review

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Synbiotics (prebiotics and probiotics) in the nutrition of critically ill patients

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ABSTRACT

Background: It is well known, nowadays, that intestinal microbiota is considered to be a synbiotic partner that maintains the host's health. Probiotics are live microorganisms and prebiotics are selectively fermentable non-digestible oligosaccharides or food ingredients that when adequately present, provide health benefit for the host. The mechanisms in which these microorganisms are involved are gastrointestinal barrier function improvement, gut flora modification by antimicrobial peptides inducted by host cells, antimicrobial factors released by probiotics, epithelial adherence competition, and immunomodulation that advantages the host. Synbiotics are a synergic combination of probiotic bacteria and prebiotic ingredients that promote the growth of the former.
Methods: In the present study, the existing evidence regarding the beneficial role of probiotics, prebiotics, and synbiotics in critically ill patients was evaluated.
Results: The results were rather encouraging about the early use of pro/pre/synbiotics in daily care of critically ill patients but still controversial due to the lack of specific supportive evidence and strain specificity.
Conclusions: Despite the positive effect of pro/pre/synbiotics supplementation, they cannot be widely applied in critical care clinical practice until well-designed prospective and randomized controlled trials are performed.

Key Words: *Probiotics; prebiotics; synbiotics; critically ill; nutrition*

INTRODUCTION

Nutrition support of critically ill patients is very important for their favourable progression. In the past, herbal medicines were almost the only possibility of therapeutic intervention, even for critically ill patients and, therefore, the gastrointestinal tract was the only route for the administration of both food and treatment. The appearance of appropriate tubes made it possible to administer nutritional factors from the rectum in patients who could not be fed per os for various reasons. This practice was quite common in the 19th and the early 20th century. Intravenous administration of nutritional solutions was a new approach in the field of perioperative nutrition, which was established in clinical practice during the mid-20th century

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[1]. During this period, great efforts were made for the development of exclusively intravenous feeding techniques for critically ill patients, as at the same time there was no interest in the use of the gastrointestinal system and natural feeding per os. Despite all these efforts, the design and implementation of a perfect, effective and safe parenteral nutrition system failed for several reasons. The effect of parenteral nutrition on the immune system and the need for intestinal mucosal barrier integrity and functionality maintenance were not evaluated as they should. The value of nutrients and antioxidants produced from the enzymes of the lower gastrointestinal tract microbial population was overlooked and the necessity to maintain the normal production of more than 100 gastrointestinal (GI) secretions, necessary for the immune system adequacy and local infection control was not evaluated [2]. Only in the last 20 years, research and biotechnology have focused on the gastrointestinal tract and the importance of microbial flora in the restoration and maintenance of health. Also, it was recognised that important therapeutic manipulations, such as antimicrobial chemotherapy and radiation were characterised by devastating effects on the immune system, gastrointestinal mucosa, and microbial gut flora. So, nowadays, several nutrient solutions for intestinal artificial feeding are produced, which, apart from the basic nutritional substrates (amino acids, carbohydrates, fats, vitamins, and minerals) in micro molar or macromolecular form, contain various ingredients such as fibers, which by fermentation from colon microbes produce nutrients both for the body (short-chain fatty acids, antioxidants) and the gut microbial flora. The intestinal bacterial system can be affected via the interaction of administered prebiotics with the symbiotic intestinal bacteria, especially the colon, where anaerobic bacteria can ferment non-absorbable dietary carbohydrates. By the fermentation, intestinal pH is reduced and that stimulates the non-pathogenic bacteria growth (prebiotic effect) and releases short chain fatty acids (butyric, acetic, and propionic acid). Butyrate is the main energy source for epithelial colon cells and inhibits Nf-kB activation. This prevents the expression of specific genes that encode cytokines promoting inflammatory response [3]. Additionally, butyrate increases the inflammatory cell apoptosis [4].

Homeostasis and intestinal tract

It is known that health and well-being are determined by the overall homeostasis of the organism, meaning maintaining balance between the physiological systems that control the entire body's functions range [5].

Modern dietetic habits differ significantly from that of Paleolithic men. Our distant ancestor annually included in his diet more than 500 plant species, while today we use less than 50. The food was usually rough and wet, while today we consume preserved, dried, or cooked foods, processes that can destroy many sensitive dietetic factors and antioxidants. It is estimated that the Paleolithic man consumed five to ten times more fiber, at least tenfold greater amount of antioxidants, fifty times more omega-3 fatty acids and even eventually million times more bacteria and fungi. The main source of food was the ground and the food was usually "infected" with microbial material. A protective layer of microbial flora covers all surfaces of the body, including the gastrointestinal tract which is important for the prevention of infections and inflammatory reactions. The gastrointestinal tract is covered by 1-2 kgs of microbial flora and the skin by about 200 gr of bacterial populations. Other important body parts of protective microbial flora are the oral cavity and pharynx, the tracheobronchial tree, and the vagina. Each one of these positions is estimated to be colonised by 20 g of microorganisms, approximately. Excessive cleanliness disrupts this defensive layer of microorganisms and subsequently, susceptibility to opportunistic bacterial infections is more likely. The animal instinct to lick their wounds is based on the salivary protective flora and growth factors that can be inoculated on the wound.

The gastrointestinal tract microbial flora has five significant functions: a) reduction or attenuation of potential pathogens, b) reduction or attenuation of various exogenous or endogenous toxins, mutagens, carcinogens, etc., c) immunological modulation, d) maintenance of the normal apoptotic process and e) release of a large number of dietary antioxidants and growth factors [6].

In recent years, the disorder of the intestinal environment (flora or mucosal barrier) was recognised as one of the main causes of allergic and autoimmune diseases and of underlying inflammation. Patients undergoing long-term medication or major surgery tend to be merely affected by this disorder. A considerable number of experimental studies attribute the increased morbidity of these conditions to bacterial migration. However, the interpretation of the different responses of patients to significant septic complications is poor. It is assumed that two factors are equally important: a) the previous capability of the immunological response and b) the increased toxicity of potentially pathogenic microorganisms which are present in patients' flora [7].

Autoimmune diseases, gastrointestinal cancer, obesity, and cardiovascular diseases, among others, are linked to derangements in composition, numbers, or habitat of the gut microbiota due to lack of probiotics-synbiotics. Resetting the gut microbiota to the prior state, despite

hardly feasible, can be achieved by the use of probiotics, as well-designed clinical studies indicate [8-10]. It is well known that intestinal microbiota play a major role in the pathogenesis of inflammatory bowel disease (IBD) and colonic cancer. Research indicates that probiotics and/or prebiotics could be used as prophylaxis in suspected or established IBD and carcinogenesis, due to their aforementioned physiologic characteristics and lack of side effects [11].

For years, it was believed that as long as the bacteria are restricted to the lumen of the gastrointestinal or respiratory system, they cannot be dangerous to the body. This view has been recently challenged by Alverdy et al [12]. It was observed that the injection of microbial culture, quantitatively and qualitatively similar to the bacteria of the intestinal tract, into the systemic circulation (intravenously, intraperitoneally or in the mesenteric lymph nodes) of healthy or under stress animals, resulted in a limited only systemic inflammatory response without organs dysfunction and was followed by speedy full recovery [12]. On the other hand, since the mid-70s, it has been found that injecting a small amount of Pseudomonas Aeruginosa culture into the cecum had fatal outcomes. This dose was much lower compared to the intravenous lethal dose [13]. It has also been observed that intratracheal instillation of the same culture of pseudomonas caused the death of all animals, which was not the case with the intravenous administration of an equal amount of the same strain culture [13].

It is known that artificial nutrition with pro/prebiotics positively influences intestinal mucosal barrier functionality, reducing infections and improving patient's clinical outcomes [14,15]. Dysbiotic microbiota has been associated with IBD and also with obesity and metabolic syndrome. Microbial manipulation (probiotic, prebiotic) impacts colorectal carcinogenesis [10]. Simren et al presented a hypothesis that abnormal microbiota activates immunological response, and subsequently increases epithelial permeability and dysregulates the enteric nervous system. These phenomena can be reversed with probiotic and synbiotic supplements administration [16].

It is also widely accepted that haematogenous infections have relatively little impact on the overall progress of the patient, the rate of organ failure, and mortality. On the other hand, the dramatic increase in the toxicity of potentially pathogenic microorganisms of the intestinal lumen or respiratory system plays a significant role in the patients' outcome. The modification of the toxicity of potentially pathogenic microorganisms of the gastrointestinal tract occurs due to the environmental changes of the intestinal lumens, caused by the disease, the lack of the foods' pH, the local redox state, the reduced osmolality and the compensatory secretion of hormones. Also under severe metabolic stress, the increase of locally secreted noradrenaline enhances intraluminal bacteria toxicity [17]. It is believed that potentially pathogenic microorganisms, which in normal conditions are inactive colonisers of the human body, alter their phenotypic characteristics under stress conditions and become pathogenic and life-threatening [1]. Alverdy et al, referring to Escherichia coli, suggested that these bacteria adhere to the host's cells for feeding reasons and cause activation of the transcription pathways in the mucosal cells, thereby causing an increase in innate immunological inflammatory response [12].

Antibiotics as therapeutic agents

Antibiotics are used to prevent and treat infections in patients with severe acute inflammatory conditions, such as severe acute pancreatitis, extensive burns, blunt trauma patients, patients with major surgery, and ICU patients. Most often this effort has only a limited impact on morbidity, mortality, and overall disease progression. In the last 20 years, selective sterilisation of the gastrointestinal tract (Selective Gut Decontamination, SGD) has been widely accepted, as a therapeutic approach to reduce the rate of septic complications and it was followed by prophylactic eradication of potentially pathogenic microorganisms of the GI tract, from the oropharynx to the rectum, without simultaneously undermining the natural anaerobic microflora [18]. In this approach, an orally administered combination of three or more non-absorbable antibiotics such as colistin, tobramycin, nystatin, gentamicin, or amphotericin B is administered alongside the same antibiotics as a local ointment for "sterilization" of the oropharyngeal cavity. The treatment is repeated every six hours for at least four-six weeks. So far, more than 30 clinical studies have been performed that were evaluated by two recent meta-analyses [19,20]. Both of these confirm a slight but still statistically significant reduction in the frequency of hospital-acquired pneumonia and an improvement in survival in certain critically ill patients. In contrast, two randomised controlled trials [21,22], did not confirm the positive effects of selective gut decontamination in liver transplant patients. In the first study, a complication rate of 32.4%, versus 27.9% in the control group was found, while in the other rates were 86% and 84.5 % respectively.

Impaired nutrition and reduced immune resistance

During the last two decades, clinical studies [23,24] indicate that 50 % of ICU patients exhibit severe malnutri-

tion and signs of immunological deduction. Thus, these patients exhibit, postoperative or posttraumatic significantly higher complication rate, leading to a significant increase in the ICU time (patients with normal nutritional status 3±2 days vs 44±36 days for heavily malnourished patients), an extension of mechanical ventilation time dependence (2±2 days vs 41±37 days), an extension of the total hospitalisation time (31±24 days vs 82±40 days), higher frequency of tracheostomy (0% vs 67%) and a greater mortality rate (0% vs 28%). Furthermore, apart from malnutrition, other factors are involved in the high ratio of septic complications in ICU patients. Such factors include reduced patient resistance due to immune suppression, advanced age and severe underlying disease, presence of prosthetic material such as endotracheal tube, venous and bladder catheters, administration of antibiotics, and other drugs that modulate the resistance to microbial infections and long-term ICU stay, favouring the risk of cross infection. Septic complications appear in four areas: respiratory system (about 30% -65%), urinary tract (about 25%), haematogenous infections (about 15%), and surgical trauma (about 8%). They usually occur after a major trauma, a major surgery, or prolonged specific medication, for example liver and stem-cell transplantation (50% -85% of patients), after major esophagus, stomach and colon surgery (20% of patients), as well as after coronary artery bypass (in about 10 % of patients) [25].

Early enteral nutrition as a therapeutic intervention

The administration of enteral nutrients and antioxidants in ICU patients is accepted as a prerequisite for the avoidance of sepsis and other complications. Because the purpose of this therapeutic intervention is to reduce the intensity of stress response and the therapeutic window appears to be extremely narrow, the administration of enteral nutrition should commence the earliest possible [22,26]. More than a hundred years ago, Andresen et al., based on clinical experience, recommended enteral administration of 200-250 ml of nutrient solution, from the time of surgery, followed by continuous administration postoperatively [27]. He believed that such intervention was safe and that in this way he could prevent postoperative paralytic ileus and contribute towards the faster recovery of patients.

Approximately 80% of total immunoglobulin amount produced by the body, is located in the lamina propria of the intestinal mucosa [28], and significant quantities, especially IgA immunoglobulin, are released into the lumen of the gastrointestinal tract. IgA biosynthesis depends to a significant extent on T - lymphocytes and various cytokines that are produced by activated lymphocytes, which mediate IgA differentiation [29]. Dietary changes, physical activity, sleep, depression, age, sex, body temperature, medications, and various diseases can affect lymphocytes functionality, immunoglobulin production, and disease resistance. Hospitalisation in ICU causes major nutritional and mobilisation changes and practically deteriorates all body functions. This fact, together with the administration of a plethora of pharmaceutical agents, causes a significant immune response reduction.

In the past, critically ill patients were treated with hypercaloric dietary load (hyperalimentation). This is currently not used. On the contrary, we are now convinced that the administration of excessive energy load, enterally or parenterally, in critically ill patients, is extremely dangerous because it seems to be accompanied by critical or fatal consequences. Today, hypercaloric diet is very rarely recommended during the perioperative period, especially during the first two-three postoperative weeks. Furthermore, the aim of positive energy coverage and positive nitrogen balance, for surgical patients with moderate metabolic stress, has lost most of its merit. Instead, the value of enteral nutrition as a regulator of the immune defense mechanism is emphasised more.

It has been recently recognised that immunological status is more important than nitrogen uptake and caloric coverage. Windson et al., compared total parenteral nutrition vs enteral feeding in patients with acute pancreatitis and found similar results [30]. In the enteral nutrition group, there was a statistically significant improvement in the APACHE II score (6 vs 8, $p < 0{,}0001$), in the levels of C-reactive protein (84 mg/lt vs 156 mg/lt, $p < 0.005$) in the levels of IgM antinuclear antibody against endotoxin and the total antioxidant status of the organism. Also, the intensity of the inflammatory response, sepsis and multisystem organ failure syndrome occurrence, and ICU prolonged stay were all statistically significantly improved in the enteral nutrition group. Shirabe et al., compared total parenteral nutrition vs enteral feeding in patients who underwent hepatectomy with no differences in nutritional parameters such as binding retinol protein levels, prealbumin, and 3-methylhistidine levels [31]. Instead, they found significant differences in immune parameters, such as the total number of leukocytes, response to phytohemagglutinin and activation of natural killer cells. Even more important, is the fact that the frequency of infectious complications in the enteral nutrition group was 8% compared to 3% of the parenteral hyper nutrition group and that was attributed to severe hyperglycaemia, and intense metabolic stress caused by the parenteral hyper nutrition.

A meta-analysis demonstrated that early (within 12 hours) enteral nutrition administration reduces the rate of surgical and posttraumatic infections, maintains the physiological antioxidant level of tissues, improves anabolic processes and wound repair and maintains the intestinal mucosa functional integrity [32]. Noteworthy are also the findings of Compan et al, that commencement of enteral nutrition administration in blunt trauma patients the first six hours after their admission to ICU, compared with the administration after the first day, not only ensures normal mucosal permeability of the intestine, but is also accompanied by a significant decrease in organ failure ratio [33]. It appears that the application of early enteral nutrition (< 6 hours) has positive effects on ongoing critically ill patients. As mentioned by Marik and Zaloga, even if early enteral nutrition does not reduce the rate of septic complications, neither does it increase it [32]. Enteral nutrition reduces intestinal complications such as bacterial overgrowth caused by starvation, and production of sIgA [34].

The synbiotics (probiotics and prebiotics) as a therapeutic agent

It is undeniable nowadays that the intestinal microbiota is considered to be a synbiotic partner that maintains the host's health. Probiotics are live microorganisms and prebiotics are selectively fermentable non-digestible oligosaccharides or food ingredients that enhance host's health. The mechanisms involved include improvement of gastrointestinal barrier function, modification of the flora by antimicrobial peptides induction by host cells, antimicrobial factors released by probiotics, epithelial adherence competition, and immune system regulation to the advantage of the host. Synbiotics consist of probiotic bacteria and the prebiotic nutrients that lead to the synergic activity of both. There are many benefits of prebiotics, probiotics, and synbiotics consumption as the whole human body is covered with a plethora of microorganisms [35,36].

Prebiotics and probiotics have proven beneficial in gastrointestinal diseases and specifically prebiotics (inulin, pectin, fructo and galacto oligosaccharides) are useful substrates for fermentation in gut contributes that contribute to the maintenance of the intestinal mucosal barrier, intestinal mucosa immunomodulation and immune defense enhancement against pathogenic micro-organisms [37-39]. Probiotics are non-pathogenic bacteria, which have the ability to adhere into the intestinal mucosa and stimulating the sIgA secretion and mucus production, regulate cytokines levels, produce heat shock proteins and defensins, activate macrophages and thus improve intestinal immune function [38,40,41]. The probiotic concept is the same on all sites of actions and routes of administration, as viable organisms have the same action independently of the site (mucosa-lined cavities such as mouth, colon, and vagina) [42].

Prebiotics

The early administration of enteral nutrition with only basic nutritional factors does not seem enough. The enteral feeding solutions should also contain fibers (prebiotics). Prebiotics help the development of the intestinal mucosa, to maintain its functional integrity, to maintain water and electrolytes balance in the body, to supply the body with energy and nutritional factors, and to increase resistance against pathogens. The human colon since birth depends on prebiotics administration for normal development and functionality. Breast milk has an extremely high non-digestible oligosaccharides concentration. Human breast milk is one of the richest among mammalians' milk in non-digestible oligosaccharides, which protect, due to immune modulation, infants who are fed with breast milk from infections and inflammations [43]. These non-cleavable oligosaccharides favour the development of the non-pathogenic microorganisms in the intestine of breastfeeding infants. Phytochemicals are compounds that are found in plants but without nutritional value and they are responsible for color, odor, taste and plant defense against various diseases. Popularly known phytochemicals with antioxidant properties and cell protection role from oxidative stress are lycopene in tomato, sulfides in leeks, onions, and garlic, isoflavones in soy, polyphenols in tea and grapes, and flavonoids in fruits and vegetables [42]. Dietary fibers are endogenous components of the cell walls of plant cells, polysaccharides, non-starch polysaccharides, lignin etc. These components are resistant to intestinal enzymes and cannot be digested. Thus, they provide non-digestible substrates for the small intestine, with no or minimal absorption and metabolism rate [44]. Non-digestible prebiotic hydrocarbons are various oligosaccharides such as pectin, beta glucans, inulin, fructooligosaccharides and galacto oligosaccharides, lactulose etc. Inulin, fructo and galacto oligosaccharides lead to the growth of beneficial bifidobacteria and lactobacilli strains in the colon and reduce the burden of potentially pathogenic microorganisms [37]. It has been found that glucan administration in blunt trauma patients, significantly reduces hospital infections morbidity [45].

Often in debilitated patients, it is not possible for several reasons to administer fresh fruits and vegetables

but there is a great range of fiber, which can be used. One of these fibers, known for its bioactivity, is pectin, which protects muous, acts as an antioxidant, transports lactobacilli in the GI tract, and is an excellent substrate for bacterial fermentation. Bananas, particularly unripe, are rich in pectin and starch. The administration of green banana (250 gr/lt food) or pectin (2 gr/lt food) was tested in Bangladesh children, fed almost exclusively with rice which suffered from severe, persistent diarrhea syndrome [46]. In both children groups the frequency of diarrhea episodes, the duration of diarrheal syndrome, the vomiting frequency and the liquid volume (oral or intravenous) required for hydration were decreased. In the study, 59% of children who were given the green banana, and 55% of children with pectin, showed significant improvement from the third day of treatment in contrast to 15% of children fed only with rice. It is suggested that at least 10gr pectin per day should be routinely administered in all ICU patients, as an antioxidant for gut protection and restoration of gut microbial flora [23].

The intake of viscous hydrocarbons and non -digestible dietary fibers can promote some positive effects on the organism, such as an increase in saturation, slow gastric emptying, decreased appetite, lowering cholesterol, blood glucose, and low-density lipoproteins and depending on their size increasing the fecal mass, reducing the intestine transit time and generally improving the whole intestine function [47-49]. Thus, they can be used therapeutically to reduce calorie intake, control body weight, reduce cardiovascular disease, and prevent constipation and possibly colon cancer [44]. In diverticulitis, inulin improved the balance between potential pathogenic Enterobacteria and species of beneficial bifidobactiria and lactobacteria, by reducing the concentration of secondary bile acids [50]. The use of a fermentable oval Plantago solution (fibers) showed similar relapse rates in ulcerative colitis such as mesalazine [51].

Prebiotics can regulate the composition of colonic flora by increasing the number of specific bacteria (bifidobacteria). They can also modulate lipid metabolism, most likely via fermentation products [37]. The chicory fructans are beta (2-1) fructo-oligosaccharides (prebiotics) that can upregulate the number of bifidobacteria in colon flora, and affect the bioavailability of minerals and lipid metabolism. This can result in reducing the incidence of bowel disease, cardiovascular disease, metabolic syndromes, and cancer [37].

In vivo experiments have shown that fructans have anticarcinogenic properties on chemically-induced precancerous and cancerous lesions in the colon. In vitro experiments on human cells have shown that inulin-derived fermentation products can reduce cellular activity of cancer cells. This can be partially explained by the reduction of exposure to risk factors, thus driving cancer cells to earlier cellular death. It can be assumed that fructans act as both a blocking agent and a suppressing agent of chemopreventive activities [52].

Despite the vegetal diversity, the modern human chooses to take 90% of his food from only 17 plants and more than 50% of caloric and protein requirements come from just eight cereal seeds. The restricted variety of diet and the modern preservation and preparation methods of food, significantly reduce the benefits of nutrition and antioxidant reagents. The diet of patients is even more limited and that's why it is strongly supported that each solution of enteral nutrition should contain fibers. Prebiotics can induce high bifidobacteria levels in the colon at all ages. Placebo-controlled intervention studies show that high bifidobacteria levels induced by prebiotics can, for example, reduce sickness events in toddlers and gastrointestinal diseases in adults and enhance immune activity in elders. Even if they are administered prophylactically, prebiotics can alter the physical course of gastrointestinal disorders [53–55].

Probiotics

Probiotics are viable microorganisms administered to humans, aiming the mucosal floras. A probiotic product, depending on its form can be a type of food, food supplement, biological or pharmaceutical product [56]. Probiotics can affect the host by differentiating the gastrointestinal flora, improving the colonic microbial balance, and actively promoting the growth of desirable bacteria [57,58].

Probiotics may be bacteria, such as Lactobacillus, Bifidobacterium, Escherichia (strain Nissle 1917), Enterococcus (E. faecium SF68), Bacillus and Streptococcus, or certain fungus, such as Saccharomyces boulardii [50]. Probiotics such as Lactobacillus rhamnosus GG, L. reuteri, bifidobacteria and certain strains of L. casei or L. acidophilus have food production usage, such as dairy production, and also exhibit possible medical use. There is a wide genetic diversity among the different lactobacilli. Most of the lactobacteria that are consumed have limited fiber fermentation capacity, limited antioxidant properties, poor mucus adherence and are destroyed mainly by gastric acid and bile. Lactobacilli, which are contained in yoghurt are well known for their ability to grow in an environment without fibers and do not seem to have strong bioactivity, so, they are selected almost exclusively for their good flavor. In opposition to that, lactobacilli which are living and growing on plants, often under very difficult circumstances, have much stronger bioactive properties. These bacilli have the

capacity to ferment fibers that are difficult to cleave. This probably explains why, eating lactobacilli from vegetables, cereals, sorrel and sourdough, as expected, exhibits much better clinical response in critically ill patients. Of great interest are the lactobacilli from seeds such as oats and rye. The number and the physiology of the bacilli in rye was studied and more than 100 species of lactobacilli were found some of which demonstrate unique bioactive properties [1].

It has been shown that probiotics are effective in various inflammatory colonic conditions, such as infantile or antibiotic-related diarrhoea, recrudescing *Clostridium difficile* colitis, Helicobacter pylori gastritis, and inflammatory bowel disease. Extracolonic diseases in which probiotics have a positive effect can be female urogenital infections, surgical site infections, allergies, AIDS, respiratory and urinary tract infection, and cancer. Even metabolic conditions have been shown to alleviate symptoms with the use of probiotics osteoporosis, obesity, and, possibly, type 2 diabetes, or even gestational allergies [14,59,60]. Preventively and therapeutically, a blend of eight probiotics has been used for diverticulitis, and others have been used in ulcerative colitis, pouchitis, or even irritable bowel syndrome for symptom alleviation [61,62].

Lactobacilli have demonstrated the ability to regulate the amount of potentially pathogenic micro-organisms, toxins, and extrinsic pathogenic factors. They can regulate the innate immunological response to pathogenic factors and microorganisms by upregulating the anabolic synthesis of growth factors and other elements of intrinsic inflammation modulation [63]. Adherence to intestinal epithelium and subsequently inhibition of pathogen's adherence and proliferation are the main mechanisms of action of probiotic bacteria. Cytokine release can be initiated by probiotics. They can also produce lactic acid and bacteriocins, which inhibit pathogen proliferation and alter the microbiota. In addition, the probiotics that produce butyric acid inhibit the adverse effects of high-protein dietary carcinogens, such as nitrosamines [58]. Sivieri et al showed the positive effect of L. acidophilus 1014 on microbial metabolism and flora composition [64].

Probiotics have been suggested to intervene in the hereditary, environmental, microbiological, and immunological factors that contribute to the occurrence of inflammatory bowel disease. Possible mechanisms include probiotic competition with or suppression of microbial pathogens, regulation of an immune response, enhancement of barrier activity, and induction of T-cell apoptosis [65]. There are several interesting studies of Crohn's disease, where the genetic modification with human genes of Lactobacillus lactis strains leads to the production of IL 10 [66,67]. In two studies, the administration of E.coli Nissle 1917 [68,69] and in a study the administration of Lactobacillus GG [70] was compared with mesalazine . Remission rates were similar to mesalazine in all of the aforementioned studies. Significant results have been obtained with the use of probiotics in patients with pouchitis. In chronic relapsing pouchitis, significant reduction in relapse rates was found by combining eight different bacteria (VSL # 3), compared with placebo [71] and further VSL # 3 use reduced postoperatively pouchitis percentage, compared with placebo [72].

In intensive care-associated conditions, such as diarrhoea associated with antibiotics consumption, ventilator-related pneumonia, and necrotising enterocolitis, various probiotic strains have been used effectively, but without a consensus on the dosages and duration of treatment [73]. However, even if they are effective in reducing these conditions' incidence, the long-term mortality rates do not seem to be significantly reduced [74].

Infection of the host has been a suspected condition concerning probiotic administration, since they are indeed live organisms. They can cause bacteraemia, and thus may induce sepsis. Despite the risk of sepsis, if considered lower than that of the pathological bacteria acquired, their use can be justified as a means of therapy [14,56]. Not all probiotics are the same and thus, they cannot cause the same adverse effects [62].

Synbiotics

Gut microbiota is closely associated with specific diet and food intake. Any change in diet can cause a chain of changes in the microbiota balance and subsequently in organ function [60]. The single or combined use of probiotics and prebiotics regulates the intestinal microbiota and by extension immunological responses [75]. Intestinal microecological disturbances (dysbacteriosis) can be treated with probiotics, prebiotics, and synbiotics used for the correction of resident normal colon microflora [76]. Combined administration of probiotics and prebiotics significantly improved intestinal flora of rats, as far as probiotic bacteria and enzymes are concerned [50]. Increasing evidence on prebiotics metabolism by probiotics and the probiotics mechanism of action in microbiota, have given the chance to specifically regulate dietary changes in specific population and disease groups [77].

Gastrointestinal glands, mucosa, and mucosa-associated lymphoid system constitute 70% of the immune system. Thus, the hypothesis of the use of synbiotics in intensive care patients for modulation of the innate immune system arises [78]. Recently, it has been exhibited that early commencement of enteral nutrition combined with synbiotics

may reduce inflammatory response, regulate intestinal immunity and help infections' prevention. Oligofructose, lactulose and galactooligosaccharides are prebiotics which can regulate the gut flora balance. Critically-ill patients can be positively affected by synbiotics, with restoration of intestinal flora, improving intestinal permeability and bacterial translocation [79]. Prebiotics, probiotics, and their combination protect and cure diseases such as diarrhoea, inflammatory bowel disease, and Helicobacter infections in postoperative patients in intensive care units [6].

Synbiotic combinations can potentially regulate the microbiota as they seem to control bacteria proliferation and the short-chain fatty acids production in human colon experimental models [80,81]. Probiotic combination (L. paracasei or L. rhamnosus) with two oligosaccharide prebiotics can increase the populations of Bifidobacterium longum and B. breve, and reduce Clostridium perfringens when co-administered. This microbial shift was associated with regulation of host metabolic pathways in lipid, glucose and amino-acid metabolism, as carbohydrates were fermented by different bacterial strains. Therefore, this fact offers considerable promise for treating inflammatory bowel disease in combination with already used anti-inflammatory and immunomodulatory agents [82]. Synbiotics can restore a beneficial predominance of Lactobacillus and Bifidobacterium species. It has been shown that selected probiotics can minimise the relapse of ulcerative colitis and pouchitis [83]. A Synbiotic product (Flortec) containing Lactobacillus paracasei B21060 was admnistered in patients with diarrhoea-predominant inflammatory bowel disease and improved pain and well-being [84]. Bifidobacteria and lactobacilli are the main beneficial probiotics contributing to lactose digestion in lactose-intolerant patients, reducing symptoms and boosting immunological and anti-inflammatory responses [85]. Several studies in animal models showed the beneficial role of synbiotics. Probiotics, prebiotics and synbiotics are protective against oxidative stress and inflammation in the terminal ileum in neonatal rats, but their efficacy may be reduced when administered during hyperoxia/hypoxia insults [86]. Prebiotic and/or synbiotic supplementation in a neonatal intestinal failure piglet model showed that they can promote the functionality of the residual intestine. Synbiotics showed higher outcomes than prebiotics alone, proving the enhanced outcomes of the synergy provided by the combination of prebiotics and probiotics [87]. Rishi et al demonstrated that L. acidophilus, inulin, and their combination can have positive effects on liver damage induced by Salmonella in a murine model. Symbiotic combination decreased bacterial translocation in the liver and levels of serum aminotransferases, denoting their therapeutic contribution to the Salmonella infection therapy in mice. Even when they were administered in healthy mice, they also showed reduced lipid peroxidation levels, increased superoxide dismutase levels and glutathione, as well as decreased nitric oxide levels. Different mechanisms could be involved in the synergic effect, whereas the probiotic alone seems to be more effective [88].

Intestinal disorders and metabolic syndromes are associated with dysbiotic microbiota development and, therefore, microbial flora regulation (probiotic, prebiotic) impacts colorectal cancer development [10]. There seems to be a significant impact of prebiotics, probiotics and synbiotics on malignancy treatment in colon cancer patients by causing bio-antimutagenic and desmutagenic effect [89,90], immune response stimulation, inflammation reduction, inhibition of tumour cells formation and decrease in bacterial enzymes which hydrolyse beta-glucuronidase and other precarcinogenic substances [91,92]. Synbiotics could have a potential in the prevention and therapy of colorectal cancer affecting gut microbiota and, that way, influencing the immune system [93]. Also, synbiotics affect metabolic pathways such as the secondary bile acids deconjugation, the activities of bacterial enzymes, as well as mineral absorption [94].

Shimizu et al, found out that in critically ill patients, synbiotics that have been reported to significantly decrease sepsis, gut flora and environment are significantly altered (maintained and repaired), and the number of synbiotic anaerobes is associated with prognosis, but did not define mechanisms of probiotic/synbiotic treatment therapeutic effect and appropriate conditions for use [95]. Patients after liver and pancreas surgery or trauma patients benefited most from synbiotics; however, synbiotic preparations need extensive testing before clinical implementation to define the exact synbiotic combination and the therapy duration [73]. Prevention of infectious complications after major surgeries such as acute pancreatitis, liver transplantation, and biliary cancer has been investigated by the use of probiotics and synbiotics as post-operative treatment and concluded in the potential clinical application [96].

The understanding of these complex interactions of microbiota and eukaryotic cells can positively affect various aspects of metabolism and immunity and can further provide the knowledge of proper manipulation of a pathologic condition, if it arises [97]. It has been demonstrated that prebiotics, probiotics, and their combination can regulate the gut flora, reduce inflammation in the colon, and potentially induce disease remission [98]. Viable bacteria can be administered in high dosages in fermented products with the proper selection of prebiotics and probiotics [99], and that is leading towards a more targeted development of functional food ingredients [76].

Clinical Evaluation of Formulations with one Lactobacillus species and one kind of fiber

Lactobacillus Plantarum (LP) is commonly found in the intestinal tract of Asian and African farmers, whose diet is mainly based on fresh vegetables, rich in Lactobacillus. There is strong evidence that Western lifestyle and diet inhibit intestinal colonisation with the LP. The Lactobacillus has been detected in 2/3 of the Seventh-day Adventists, North Americans who are almost exclusively vegetarian, and only in 1/4 of North Americans with Western diet [100]. The three main types of lactobacilli isolated from jejunal biopsies in a Swedish population, are the L. Plantarum (24%), the L.Rhamnosus (12%) and L.Casei subtype of Pseudoplantarum (in 10%) [101]. Furthermore, L.Plantarum was identified in 1/3 of infants aged 3-8 weeks [102].

A team of Lund University researchers analysed the beneficial effects of synbiotics in the preparation of a specific enteral feeding solution consisting of oatmeal that was fermented by strain 299 of L. Plantarum [40]. This strain was shown to have the ability to ferment oats and without being affected by gastric fluid and bile [103]. The enteric solution based on oats and L. Plantarum 299 was tested in 3 groups of critical conditions: liver transplantation, severe pancreatitis, and recent major gastrointestinal surgery.

In 2002, the efficacy of a synbiotic mixture in patients who had undergone liver transplantation and postoperatively (from the second postoperative day) were administered early enteral nutrition was evaluated in a prospective randomised study [104]. Patients were divided into three groups. In the first, 32 patients underwent selective gut decontamination for 6 weeks before surgery. In the second, 31 patients received a specific solution of live L. Plantarum 299 strains at a dose of 10^9 with 15 gr fermented fiber for 12 days postoperatively. In the third group, 32 patients were treated for the same period (12 days) with the same special solution, but with heat-inactivated L. Plantarum. There was no postoperative mortality, although there were 23 postoperative infections in the group of selective gut decontamination, four in the group with living lactobacilli and 17 in those with inactivated lactobacilli. Clinical infection signs presented at 15 out of 32 patients in the first group (48%), four of the 31 in the second (13 %) and 11 out of 32 in the third (34 %) ($p = 0.017$). The most frequent postoperative infection was cholangitis, in ten, two and eight patients, respectively, and pneumonia in six, one and four patients from each group. The microorganisms most frequently isolated were enterococci, in eight, one and eight patients and staphylococci in six, one and three patients. E.coli or Klebsiella was not isolated in any of the second group patients Non-infectious complications were observed in 15 first group patients, 16 in the second and 19 in the third. Finally, early graft failure manifested in 10, 10 and 15 patients, respectively, and hemodialysis was necessary to eight, two and four patients of each group and 12 patients totally were operated again, six, four and two patients in each group. The CD4/CD8 ratio was better in the living Lactobacillus group ($p = 0.06$) as well as the duration of antimicrobial chemotherapy administration and the time spent in the ICU and the total time of hospitalisation, without any statistical significance of these differences.

Regarding the clinical outcome of patients with acute pancreatitis, infection of pancreatic necrotic tissues is an adverse prognostic factor, which causes a significant increase in morbidity and mortality [105,106]. Almost one week after the invasion of severe pancreatitis, pancreatic necrotic tissues are infected in 25% of patients and three weeks after 75% of the patients are infected. All therapeutic manipulations, including the administration of antibiotics and various inhibitors of cytokines that promote inflammatory reaction, failed to significantly ameliorate the progression of these patients [107-109].

In a study from Gyor in Hungary, 45 patients with severe necrotising pancreatitis were divided into two groups [110]. In the first group of 22 patients, an enteral nutrition formulation containing 10^9 live organisms of L. Plantarum strain 299 and substrate 10 gr oat fiber was administered by nasojejunal catheter twice a day for one week, The remaining 23 patients received the same enteral formulation, but the lactobacilli were inactivated by heat. Contamination of pancreatic necrotic tissues occurred in 4.5% of patients in the first group (1/22) and 30% (7/23) of the second. ($P = 0,023$). In addition, patients in the first group had shorter hospitalisation duration (13, 7 days vs 21,4 days), but not statistically significant, probably due to the small number of patients.

Lahner et al studied Lactobacillus paracasei B21060 and high fiber diet in symptomatic uncomplicated diverticular disease in a randomised, multicenter, controlled study for 6 months. Patients, aged 40-80 years, were divided into two groups. The first (24 patients) received synbiotic Lactobacillus paracasei B21060 (Flortec) once daily with high-fiber diet for six months, and the second (21 patients) received only high-fiber diet for six months. In both groups, abdominal pain was significantly decreased after six months, but in the symbiotic group the proportion of patients with less was higher. Abdominal bloating was significantly decreased in the symbiotic group, but not in the second group. Thus, a high-fiber diet is effective in decreasing abdominal pain in symptomatic diverticular disease and by the combination of high-fiber

with certain synbiotics, abdominal pain and bloating can be significantly enhanced [111].

Enteral nutritional solution with live strains of L. Plantarum 299 and fermented oat fiber was used in a randomised study of patients who had undergone major surgery in the gastrointestinal system, and was compared either with the same composition but with heat-inactivated strains L. Plantarum 299 or by standard enteral nutritional solution [104]. The study included 90 patients, of whom 29 had undergone hepatectomy, 26 pancreatectomy, 22 gastrectomy, 9 colectomy and 4 intestinal bypass. The patients were divided into 3 groups, which were comparable to the number, surgery severity and metabolic and hemodynamic parameters. It was found that within one month of the study initiation, septic complications appeared at three out of 30 (10 %) patients in each group receiving either live or inactivated strains of L.Plantarum, and at 9 out of 30 (30 %) of patients in the group receiving the usual enteric artificial diet (p = 0.01). Noteworthy was the fact that the largest difference occurred in the rate of hospital pneumonia (six patients in the group of normal enteral nutrition, two in the group with live strains and one in the group with inactivated strains of L.Plantarum). The protective effect of that specific dietary solution was more evident in patients who had undergone gastrectomy or pancreatectomy. In the first group, with live strains of L.Plantarum, septic complications occurred in one of 15 patients (7 %), in the second, with the inactivated strains, in three of 17 (18 %) and in the third, with the usual nutrient solution, in eight of 16 (50 %) patients. In the first group of patients, the antibiotic dose administered was also reduced (p = 0.04) as well as the duration of antimicrobial chemotherapy (4±3.7 days, 7±5.2 days, 8±6.5 days, respectively for three patient groups). Non-infectious aetiology complications presented in 13%, 17% and 3% of each group, respectively. Additionally, there were no significant differences in hemoglobin value and the number of leukocytes, in the value of C- reactive protein, blood urea, bilirubin, albumin, total white blood cells, CD45RA, CD45RO, CD4, and CD8, of lymphocytes and natural killer cells and of CD4/CD8 ratio. Finally, there was no difference in total time of hospitalisation.

In another clinical study including surgical patients, the efficacy of fruit juice solution and synbiotic agents (PROVIVA) was evaluated. The probiotic factor was similar (L.Plantarum 299 V), but not completely the same as the L. Plantarum 299 [112]. The number of lactobacilli and oat fiber was also significantly lower than that in the previous study. In this study, the evaluated solution contained 5% mixture of lactobacilli - fermented oat fiber and 95 % fruit juice. The total content in L. Plantarum 299 V was approximately 10^7. The formulation was administered for a longer period of time than in the previous study, and at least for one week before surgery. The formulation PROVITA was administered in 64 patients and 65 were treated with the usual preoperative and postoperative care. In patients of both groups, along with the induction of anesthesia, a single dose of cefuroxime and metronidazole was administered IV. No differences were noted between the two groups in bacterial translocation (12% vs 12%, p = 0,82), the colonisation of the stomach from enteric microorganisms (11% vs 17%, p = 0,42), and septic complications morbidity (13% vs 15%, p = 0,74).

These studies differed in certain elements. The first study included patients that had undergone more severe surgical interventions with a higher risk of surgical complications. This is due to the fact that in the first study, patients in the control group (who didn't receive the synbiotic regimen) presented septic complications in a percentage of 30% (which was 50% in the cases of pancreatectomy and gastrectomy), in contrast with the patients of the control group in the second study, which included mainly patients who had undergone colectomy, and who presented septic complications in 15%. So it seems that the beneficial effect of the synbiotic regimen is more obvious in patients with a greater chance of septic complications. Moreover, in the second study, the synbiotic regimen contained lower doses of prebiotics and prebiotics. Lactobacilli quantity equal to or less than 10^7 is considered inadequate for the expression of probiotics' beneficial actions. Finally, the two studies used different types of L. Plantarum.

Clinical Evaluation of Formulations with a combination of different lactobacilli species and different types of fibers

There is evidence that the combination of different lactobacilli types with different fermentable fibers likely exerts stronger synbiotic actions. Lactobacilli growing in certain plants show different bioactivity, as natural selection results, because of their ability to fermentate these plants' fibers. Plant biologists from the University of Lund studied ecologically cultivated rye plants and found the existence of more than 180 lactobacilli species, capable of clinical use [45]. In addition, from the human gastrointestinal tract 355 other species of lactobacilli were isolated [113]. It was found that all these lactobacilli types tend to adhere to mucus, to express cell surface hydrophobicity and to adhere to collagen, fibronectin and other extracellular mesothelial tissue proteins. Eight species of lactobacilli with these properties were chosen for further

study. These species are not destroyed by exposure to 20% bile solution for an hour and PH 2,5 for two hours and were able to use as the only energy substrate inulin or amylopectin in the in vitro cultures.

Three of these species produce extra beta- galactosidase, an enzyme which is known to treat lactose intolerance symptoms. Some other species produce substances bioactive against gram-positive bacteria and Helicobacter pylori. In addition, after exposure to PH 5 for an hour, they were producing protein derivatives which showed cross-reactivity to stress proteins. Finally, four of the eight lactobacilli species that were studied, were able to transcript the nuclear factor kB (NFkB) in the nucleus of macrophages V937, and that resulted in the production of cytokines that promote the inflammatory response (IL- 1b, IL-8), antiinflammatory cytokines (IL-10) and antioxidants equivalent to 100 μg of vitamin C[229]. Based on these properties, four out of the 8 lactobacilli species were selected and used to create synbiotic formulation called Synbiotic 2000. This formulation consists of 1010 from each of the 4 lactobacteria: Pediococcus pentoseceus 5-33:3, Leuconostoc mesenteroides 32-77:1, L.paracasei subsp. Paracasei 19 and L. planetarium 2362 (probiotics), and 2,5 gr from each of four fermented fiber: b-glycan, inulin, pectin and non-digestible starch (prebiotics). All species of lactobacilli, except L.paracasei, are derived from rye plants.

In a clinical study involving 10 patients with severe chronic distal colitis, enemas with Synbiotic 2000 were performed twice daily and for two weeks [114]. The study was completed with 9 patients. A significant reduction in bowel movements was recorded (2,5±38 to 1,13±0,13 on day 7 p <0,05, to 1,13±23 on day 14 p <0,05, and to 0,75±0.25 on day 21, a week after discontinuation of Synbiotic 2000, p <0,01). The number of bloody stools was also decreased (from 2±0,27 to 1±0,38, p <0,05, and to 1,12±0,35, p <0,05, on the 14 and 21 days respectively). Furthermore, the bowel movement frequency during the night and the emergency bowel movements were decreased and the stool consistency was improved. Administration of Synbiotic 2000, was well tolerated, without major side effects except for mild bloating and increased gas excretion in two patients.

In a randomised double-blind study including 66 patients who had undergone liver transplantation, the efficacy of Synbiotic 2000 was compared to the administration of 4 types of fibers contained in this formulation [115]. The administration began one day before transplantation and continued for 14 days postoperatively. One patient from the group of Synbiotic 2000 presented clinical signs of infection, in contrast to 17 of the 33 (51%) patients in the fiber group.

A certain synbiotic formula (Synbiotic 2000Forte) has been shown to improve sepsis rates in critically ill intubated multiple trauma patients, thus reducing the needed time for intensive care treatment and mechanical support, with lower intestinal permeability and fewer infections [116,117].

Macrophage activation by GI tract endotoxins is believed to increase TNF- a, which may contribute to the progressive liver destruction in cirrhosis. Furthermore, overexpression of blood monocytes Toll-like receptors four and two is significantly related to increased TNF- a production after stimulation by endotoxin and gram-positive microorganisms. If synbiotics can reduce Toll-like receptors expression and decrease TNF –a production, then there could be a safe and inexpensive solution, for long-term management of liver diseases under evolution. This hypothesis can be strengthened by the study that showed that Synbiotic 2000 administration in 8 of 11 cirrhotic patients reduced by 54 % the TNF-a blood monocytes production after stimulation by endotoxin or intestine - toxin b of staphylococcus aureus [118]. Synbiotic 2000 was well tolerated without side effects and its action on chronic liver disease was also evaluated in a double-blind study with 55 patients [115]. 3 groups were studied: first, Synbiotic 2000 administration, (20 patients), second, only fiber of Synbiotic 2000 administration (20 patients) and third, administration of non-fermented fiber (placebo) (15 patients). The administration of a combination of lactobacilli and plant fibers for one month significantly increased the number of intestinal flora lactobacilli. A similar increase was not observed in the second and third groups. In the non-fermented fiber group, the stool PH decreased significantly between 6.5 and 7, while the other two ranged between 5 and 5.5. In the first two groups the Escherichia coli, Staphylococcus and Fusobacterium populations in stool were significantly reduced, but pseudomonas and enterococci populations did not. The ammonia value in serum decreased significantly in the Synbiotic 2000 group, (from 60,5+2,9 to 38,6+3,9 μmol/lt) and in the fiber group (from 63,6±3,9 to 41,5±5,2 μmol/lt), but not in the placebo group (from 60,5±2,9 to 58,6±3.9 μmol/lt). Also, in the first two groups, the endotoxin value, as well as the alanine transferase level were lower (from 252±182 to 84±65 U/lt, p <0,01 in the Synbiotic 2000 group, and 110±86 U/lt, p <0,05 in the fiber group). A similar reduction of these two parameters was not recorded in the placebo group. Finally, significant improvement was noted in the first two groups of patients in the psychometric tests results, as well as in the frequency and severity of hepatic encephalopathy.

Yokoyama et al evaluated the effect of administrating

synbiotics perioperatively versus no administration on bacterial translocation to mesenteric lymph nodes (MLNs) and the manifestation of bacteraemia postoperatively after oesophagectomy in a randomised clinical trial. 42 patients with oesophageal cancer were included in the study and divided randomly in synbiotics or no synbiotics (control) groups. MLNs were taken from the jejunal mesentery before dissection (MLN-1) and after the digestive tract was restored (MLN-2). Blood and feces samples were collected pre- and postoperatively. Microorganisms from blood and feces preoperatively and postoperatively were detected using a bacterium-specific ribosomal RNA-targeted reverse transcriptase–quantitative polymerase chain reaction method. Microorganisms were detected more commonly in MLN-2 samples in the control group than in the study group (P =0.035). In addition, bacteraemia on the first postoperative day was more frequent in the control group than in the study group P =0.025). Neutrophil counts on first, second and seventh postoperative days were all significantly lower in the study group than in the control group. It was concluded that perioperative use of synbiotics reduces bacteraemia and mesenteric lymph nodes bacteria incidence, reducing the inflammatory response and providing a more uneventful postoperative course after surgery for esophageal cancer [119].

In a randomised controlled trial, the administration of the symbiotic combination of inulin, oligofructose and Lactobacillus rhamnosus and Bifidobacterium lactis was studied in patients after polypectomy. They were compared to patients with colon resection only and the results were that the symbiotic use slightly stimulated the systemic immune system [120].

The aforementioned symbiotic combination has been shown to significantly reduce necrosis in colonic cells due to fecal water deregulation and ameliorate the function of epithelial barrier in patients with polypectomy. In addition, it can regulate the flora by increasing Bifidobacterium and Lactobacillus and decreasing Clostridium perfringens [102].

Sugawara et al studied the impact of synbiotics administration perioperatively in biliary cancer patients involving the hepatic hilus undergoing high-risk hepatobiliary resection. Patients were randomised into two groups; in the first, patients received postoperative enteral feeding with synbiotics, while the second group received pre- and postoperative synbiotics. Lactulose-mannitol ratio, serum diamine oxidase activity, natural killer cell activity, interleukin-6, fecal microflora, fecal organic acid concentrations, and complications were determined pre- and postoperatively. Lactulose-mannitol ratio and serum diamine oxidase activity had similar changes in both groups. Preoperatively in the second group, natural killer cells activity, and lymphocytes increased, while interleukin-6 reduced significantly (P =0.05). Serum interleukin-6, white blood cell counts, and C-reactive protein postoperatively in the second group were significantly lower than in the first group (P =0.05). Preoperatively, fecal cultures showed significantly increased numbers of *Bifidobacterium* colonies in the second group (P =0.05). The second also had significantly higher total organic acid concentrations in feces postoperatively than the first (P =0.05). Postoperative infectious complications were recorded in 30.0% of patients in the first group and 12.1% in the second (P =0.05). This study concludes that immune and inflammatory responses can be reduced by preoperative administration of synbiotics, and this can lead to decreased postoperative complications after surgical treatment for biliary tract cancer [121].

In a randomised double-blind, placebo-controlled trial, eighty patients following pylorus-preserving pancreatoduodenectomy (PPPD), received enteral nutrition immediately postoperatively. The patients were randomised into two groups, in the first, they were administered a compound of four Lactobacilli and four fibers and in the second, the placebo group, received fibers only one day preoperatively and for eight days after the PPPD. In the first group, postoperative bacterial infections were significantly less (12.5%) than in the second one (fibers only, 40%). Moreover, the fibers-only group received antibiotic therapy for a shorter period [122].

In another double-blind randomised placebo-controlled trial, 68 patients, in which colorectal surgery was performed, were divided into three groups and the systemic inflammatory response was studied. In the first group, 20 patients received a synbiotic combination (multi-strain/ multi-fiber Synbiotic2000) that consisted of four lactobacilli and four prebiotics. In the second group, 28 patients received prebiotics and heat-deactivated probiotics (lactobacilli) and in the third group, 20 patients received preoperative mechanical bowel cleaning only. Values of interleukin-6 and fibrinogen were significantly higher postoperatively in the synbiotic group. It was concluded that the use of prebiotics in colorectal surgery has a similar protective anti-inflammatory effect as mechanical bowel cleaning [123].

On the other hand, another randomised clinical trial showed no measurable effect on bacterial translocation, gastric colonisation, systemic inflammation, or sepsis in elective abdominal surgery after the administration of probiotics of five different probiotic species combined with the prebiotic oligofructose [124].

Few studies have measured the synbiotics effects on

critically ill patients, but their initial findings are very promising [1]. Early enteral feeding with synbiotics could hamper late complications in severe acute pancreatitis [110]. The routine treatment protocol of severe acute pancreatitis seems that can be benefited from the addition of synbiotics in the early enteral feeding [125]. However, a recent meta-analysis showed that prebiotics, probiotics or synbiotics treatment shows no significant improvement in patient outcome with acute pancreatitis [126]. The use of probiotics remains controversial and furthermore, the administration of the probiotics is not without risk, as demonstrated in critically ill patients with severe pancreatitis treated with probiotics in combination with fiber-rich enteral nutrition 2 times daily, resulting in non-obstructive necrosis of the small intestine [39,127]. In conclusion, the beneficial roles of synbiotics are summarised in Table 1.

Conclusions- Prospects

Although the exact mechanism of the beneficial clinical effects of synbiotics is not yet fully known, most of the existing evidence supports the hypothesis that are due to the important properties of both prebiotics and probiotics factors.

To this day only the bioactive properties of a limited number of fibers have been studied. We need to explore new and highly bioactive plant fibers which are in nature in great abundance. We should determine and define probiotics that could be used as a substrate for specific dietary factors production, such as glutamine, arginine and polyphenols. Until now bioactivity of a few lactobacilli was studied. It is required thus to study even more probiotic bacteria. As lactobacilli grow on plants whose fibers are mainly used for fermentation, research should be focused on plants with known substances beneficial for the body. We have to focus on new synbiotic combinations of prebiotics and probiotics with beneficial action.

So far, the synbiotic effects have been investigated in patients with severe pancreatitis, colitis, Crohn's disease, cancer, major surgery, burn victims and liver transplantation. The research should be expanded into other critically ill patient groups, such as those with stem cell transplants.

In the preoperative preparation of the colon, the potential benefits of administration of synbiotics, against the use of antibiotics or the mechanism of colon cleansing should be investigated. The synbiotics can reduce the levels of fibrinogen and activator of plasminogen -1, and thereby they can increase fibrinolysis. Thus, their therapeutic skills in treating thrombosis should be under investigation.

As the sufficient administration of prebiotics and probiotics appears to strengthen the endogenous immune

TABLE 1. Beneficial roles of synbiotics

Prophylaxis in suspected or established IBD and carcinogenesis
Positive effect in various inflammatory colonic conditions
Enhancement of Intestinal Mucosal Barrier functionality
Maintenance of intestinal mucosa immunomodulation and immune defense enhancement against pathogenic micro-organisms
Reduction of septic complications in ICU
Modification of the flora by antimicrobial peptides induction by host cells
Antimicrobial factors released by probiotics
Epithelial adherence competition
Reduced inflammatory response
Reduced cardiovascular disease rate through lipid metabolism regulation
Enhancement of intestinal mucosa development, functional integrity maintenance, water and electrolytes balance maintenance, increased resistance against pathogens
Immune system regulation to the advantage of the host
Regulation of intestinal immunity and infections' prevention
Calorie intake reduction and body weight control
Effect on metabolic pathways such as the secondary bile acids deconjugation, the activities of bacterial enzymes as well as mineral absorption
Reduction of levels of fibrinogen and activator of plasminogen -1, and increase of fibrinolysis
Restoration of intestinal flora, improved intestinal permeability and bacterial translocation

system, and the long-time administration is not accompanied by side effects, the effectiveness of synbiotics in patients suffering from endemic chronic diseases such as atherosclerosis, cancer, diabetes, chronic pulmonary diseases, liver diseases, inflammatory bowel diseases, HIV, cystic fibrosis, and hemodialysis patients should be further explored.

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Review

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The role of autofluorescence and Indocyanine green (ICG) in Endocrine Surgery

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ABSTRACT

Parathyroid preservation during total thyroidectomy is crucial in preventing postoperative hypoparathyroidism. The use of intraoperative parathyroid hormone monitoring has been shown to reduce the incidence of hypoparathyroidism. However, this method has limitations, and alternative techniques such as autofluorescence and ICG angiography have emerged as potential options for parathyroid preservation. The use of ICG angiography and near-infrared autofluorescence has shown promise in improving parathyroid gland preservation and reducing the risk of postoperative hypocalcaemia and hypoparathyroidism. The combination of autofluorescence and ICG angiography has also been found to be useful in identifying parathyroid glands in challenging cases, such as reoperations for thyroid or parathyroid diseases. However, further studies are needed to establish its long-term safety and efficacy and to address the limitations of its implementation in clinical practice.

Key Words: *Parathyroid glands; hypoparathyroidism; autofluorescence; indocyanine green; thyroidectomy*

INTRODUCTION

Total Thyroidectomy (TT) is one of the most commonly performed operations in Endocrine Surgery. TT is a procedure which has evolved tremendously, zeroing out historically reported mortality and significantly reducing morbidity making it a safe although technically demanding surgical procedure associated with excellent postoperative and oncological outcomes, if performed by experienced surgeons. However, when complications do occur, they can become life threatening or have a major impact in the quality of life of the patients. The incidence of surgical pathology of the thyroid gland has led to the ability of endocrine surgeons to accumulate significant experience in surgical technique. In this context, endocrine surgeons must strive for even better outcomes in complications such as injury of recurrent laryngeal nerve and transient or permanent postoperative hypoparathyroidism, which still remains a challenge even for high volume endocrine surgeons [1,2].

The identification and vascular preservation of the parathyroid gland vasculature is a difficult, alas crucial step of TT. More specifically, this step is characterized by obvious difficulties such as identification of the parathyroid glands among the surrounding tissues and their highly fragile and submillimeter vessels which cannot be easily identified or preserved. In addition to this, variations in location (e.g. intrathyroidal parathyroid gland, intrathymic parathyroid gland, ectopic superior parathyroid gland), morphology and blood supply make the intraoperative identification of parathyroid glands demanding even with high expertise and experience. Unintentional injury of parathyroid glands and their vascularity or removal of parathyroid glands with surgical specimen during TT, may lead to transient or permanent hypoparathyroidism. Unfortunately, the result of permanent hypoparathyroidism, apart from the life-long dependance on calcium and Vitamin D supplementation, may severely impact the quality of life, the

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ability to work and also predispose to renal impairment and chronic kidney disease, which in turn increases the morbidity and mortality rates for these patients.

In recent decades, strategies for the assistance in identification and preservation of function of parathyroid glands during TT has been a field of intense research, in order to reduce the rate of postoperative hypocalcaemia (temporary and permanent). In the early 1970s, methylene blue was used for the first time as a means of identifying pathological and normal parathyroid glands, but its use did not show any benefit in relation to their simple intraoperative identification [3]. Additionally, the methylene blue technique had some potential complications at the site of its injection, such as neurotoxicity. In 2006, aminolevulinic acid, an agent that acts as a photosensitiser and accumulates in the parathyroid parenchyma was used, but without significant results [4]. Hyun et al described that some synthetic fluorescent agents may be useful for identifying parathyroid glands in animal models. Most of these agents and methods did not have significant results and were not applied in clinical practice [5].

Recently, some innovative techniques using near-infrared autofluorescence (NIR-AF) appear to be able to help overcome current limitations in locating and preserving parathyroid glands.

The use of Autofluorescence in Endocrine Surgery

Autofluorescence is the natural emission of light from intrinsic fluorophores. The term is used to distinguish light originating from artificially added fluorescent agents (exogenous contrast agent) such as indocyanine green (ICG). The application of this phenomenon to identify the parathyroid glands using near-infrared (NIR) wavelengths during thyroidectomy or parathyroidectomy is the most modern technique in the field of Endocrine Surgery.

In 2011, Paras and colleagues described the property of parathyroid gland autofluorescence in the near-infrared light spectrum and their ability to emit light at a peak wavelength of 820 nm when illuminated with light at 785 nm [6]. They showed that parathyroid glands acquire the strongest autofluorescence intensity at 820 nm, in contrast to the thyroid gland which has a lower intensity. Analysing the above data and results, they concluded that parathyroid glands can be distinguished from the surrounding tissues (adipose tissue, lymph nodes, muscles, trachea, thyroid gland, nerves) using this technology [6].

While several possible fluorophores have been proposed that may be responsible for the autofluorescence of the parathyroid glands, to date, none of them have been proven. Porphyrins produce the most persistent fluorescence in biological tissue, but have a peak emission of 600–700 nm, i.e. significantly lower than that of parathyroid glands [6]. Melanin is known to be the major fluorophore in the skin, but is not found in the parathyroid or thyroid gland [7,8]. The calcium-sensing receptor (CaSR) has been suggested as the most likely fluorophore in the parathyroid glands because of its presence and its high concentration in them, however, this has not been proven [6].

The use of near-infrared autofluorescence (NIR -AF) during Total Thyroidectomy

In 2014, McWade et. Al. reported the identification of parathyroid gland autofluorescence in patients during TT, by using the modified NIR -AF imaging system, for the first time [9]. The NIR -AF image was obtained by attaching an 808 nm long-pass filter in front of the camera, which blocks the laser light at 785 nm, while allowing the emitted NIR light to pass through. According to this study, intraoperative imaging of the parathyroid glands was applied to six patients (three patients diagnosed with primary hyperparathyroidism, two with nontoxic multinodular goiter, and one with papillary thyroid carcinoma) with an average time of imaging of six minutes. The NIR-AF technique was successful in 100% of the parathyroid glands regardless of the indication of surgery, normal parathyroids in patients undergoing thyroidectomy had a statistically significantly higher signal than affected/hypercellular parathyroids in patients undergoing parathyroidectomy [9].

The majority of NIR -AF devices designed and used in Endocrine Surgery have similar technical characteristics and are developed in a similar manner. The team of Palazzo and DiMarco from the United Kingdom described their experience of using the Fluobeam800 (Fluoptics, Grenoble, France) in 365 patients (96 parathyroid and 269 thyroid) [10,11]. More specifically, the camera is connected to the Fluobeam processor which is connected to a laptop that hosts the Fluosoft software and displays the image on its screen. The camera head is placed in a sterile case for use in the surgical field. After preparation and mobilisation of one lobe of the thyroid gland has been performed in the usual manner, the surgeon uses the NIR -AF camera head in its sterile case approximately 20 cm above the "target" area of the surgical field they want to be depicted. Then, the operating lights and all background lights should be turned off. The generated images are displayed on the screen, so the surgeon must correlate the surgical findings with what is displayed on the screen. The importance of this technology during thyroidectomy is to confirm and/

or identify the parathyroid glands which are preserved in the thyroid bed after TT and to confirm a possible subcapsular parathyroid gland which is at high risk of inadvertent removal with the surgical specimen, and prevent this removal making it suitable for parathyroid autotransplantation [10,11]. In the study conducted by DiMarco et al., a total of 257 (90.5%) parathyroid glands were identified using autofluorescence, while only three glands were not identified at all in autofluorescence. One hundred seventy-three parathyroids showed high signal using autofluorescence, 61 indicated medium and 23 low signal. A statistically significant negative correlation was found between blood calcium levels and signal intensity using autofluorescence ($p<0.01$).

Indocyanine green (ICG) angiography

ICG is an amphiphilic tricarbocyanine dye with a molecular weight of 751.4 Daltons, with a maximum absorption spectrum of 805 nm and re-emission at 835 nm. Immediately after its intravenous administration, it binds to plasma proteins and circulates only within the intravascular space [12]. This process allows ICG to act as a real-time intravascular contrast agent, with a half-life of 3-5 minutes, and elimination after 15-20 minutes from the time of absorption. ICG is finally excreted by the biliary system [13].

In 2015, Suh et al. first described the use of ICG and NIR-AF for the visualisation of parathyroid glands in dogs [2]. More specifically, they described the autofluorescence intensity response according to the administered dose of ICG and showed that the method could determine the optimal concentration of ICG for parathyroid imaging in experimental animals [2]. Angiography using ICG as fluorescent dye can be performed in patients undergoing thyroidectomy to visualise the vessels of parathyroid glands that have been previously identified by autofluorescence [12]. Several studies have described that patients who had at least one parathyroid gland with adequate perfusion maintained an adequate parathyroid function postoperatively, as well. ICG angiography allows a direct assessment of parathyroid gland feeding vessels, which are at high risk of injury during TT. In addition, it can play a crucial role in decision making about whether or not a parathyroid gland should be autotransplanted after TT [12]. Although the results of using ICG autofluorescence for the identification of normal parathyroid glands are promising, several limitations have been found, because parathyroid glands are in close relation to the thyroid gland, and thus their clear distinction is not always possible, as ICG is also absorbed by the thyroid gland.

Bibliographic data on the techniques of autofluorescence and the use of intraoperative ICG angiography during Total Thyroidectomy

In recent years, numerous studies from the majority of high-volume centers of Endocrine Surgery centers have been published, comparing and evaluating both the capability of each available device, as well as the contribution of these techniques-devices to the reduction of postoperative rates of short-term hypoparathyroidism after thyroidectomy.

Considering the meta-analysis conducted by Kim et al., the use of NIR-AF is a valuable intraoperative tool for identifying parathyroid glands during both thyroidectomy and parathyroidectomy [14]. Among a total of 17 studies that have been reviewed and analyzed, authors highlighted that NIR-AF can provide high identification rates of parathyroid glands, regardless of the type of device (probe based or image-based device). However, NIR-AF can produce false-negative and false-positive results, as the detection of parathyroid glands located in deeper layers and their dissection from the surrounding tissues are quite demanding. For this reason, the classic surgical approach for finding the parathyroid glands during thyroidectomy cannot be replaced by using NIR-AF and ICG [14].

With regards to the use of these promising techniques and the prevention of postoperative hypocalcemia after thyroidectomy, the meta-analysis conducted by Barbieri et. al., stated that patients who underwent TT with NIR-AF and ICG had significantly lower rates of short-term postoperative hypocalcaemia, compared to patients who underwent classical thyroidectomy without the use of further technologies and devices [15]. Regarding long-term hypocalcaemia and short-term hypoparathyroidism, there were insufficient data on whether autofluorescence and ICG reduce their incidence rates. What NIR-AF has proven to offer is the ability to review the surgical specimen in order to rule out a possible inadvertent parathyroidectomy and the ability to autotransplant an excised parathyroid gland. The possibility of autotransplantation is certainly an important aid to the surgeon and his constant concern of reducing the rate of postoperative hypoparathyroidism [15].

Finally, in 2020, the PARAFLUO randomised controlled trial was published, in which 245 patients were studied who were divided into two groups: patients who underwent classical thyroidectomy and patients who underwent thyroidectomy using NIR-AF. PARAFLUO study highlighted that the NIR-AF group had a higher rate of identification and preservation of parathyroid glands and a lower rate of postoperative hypocalcaemia [16].

In conclusion, although all published meta-analyses consist of heterogeneous studies with poorly stratified patients with respect to the indication for thyroidectomy, they certainly depict a potential benefit in preserving the parathyroid glands after their safer identification using autofluorescence and ICG angiography, which so far has not replaced the classic technique of thyroidectomy.

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Review

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Molecular differences in colon cancer according to location: A literature review

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ABSTRACT

Differences in clinical presentation, epidemiology, prognosis, and molecular mechanisms between left-sided colon cancer (LCC) and right-sided colon cancer (RCC) have been widely studied in recent years. Indeed, mutations seen in LCC differ in nature and frequency compared to LCC. Furthermore, the differences in the biological environment, including histopathological, microbiological, and biochemical differences of the two regions promote different gene expressions in carcinomas. These molecular differences distinguish the nature of colorectal cancer according to the primary site of formation. In this narrative review, all such differences have been explored in detail with the aim of providing further insights into the topic, since in the era of individualised treatment, sidedness is a major factor in the treatment of colorectal cancer.

Key Words: *Colorectal cancer; primary tumour location; sidedness; molecular differences*

INTRODUCTION

Colon cancer, otherwise known as colorectal cancer (CRC), is the third most common cancer (after lung and breast cancers) and the second most common cause of death associated with cancer worldwide, regardless of gender [1]. The number of deaths caused by CRC has been decreasing in the last decade, possibly due to early diagnosis with screening programs and novel therapeutics [2,3]. Nevertheless, the disease continues to be of paramount significance in the field of oncology. Colorectal cancer can be caused by both genetic disorders (as in hereditary colon cancer), or environmental factors (as in sporadic colon cancer) [4]. Sporadic colon cancer, consisting of approximately 95% of all CRC cases, is associated both with genetic predisposition or gene mutations, as well as other risk factors, including some related to lifestyle such as obesity, lack of exercise, diet, smoking and alcohol

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ABBREVIATIONS: *ALK: Anaplastic lymphoma kinase, APC: Adenomatous Polyposis Coli, BA: Bile Acids, BMPR1A: bone morphogenetic protein receptor type 1A, BRAF: v-raf murine sarcoma viral oncogene homolog B1, BRCA1: BReast CAncer gene 1, CDH1: Cadherin-1 or Epithelial cadherin (E-cadherin), CMS1: Consensus Molecular Subgroup 1, CMS2: Consensus Molecular Subgroup 2, CMS3: Consensus Molecular Subgroup 3, CMS4: Consensus Molecular Subgroup 4, CRC: Colorectal Cancer, DCA: Deoxycholic acid, EGFR: Epidermal Growth Factor Receptor, KRAS: Kirsten rat sarcoma virus, LCA: Lithocholic acid, LCC: Left-sided Colon Cancer, mCRC: metastatic colorectal cancer, MLH1: MutL homolog 1, MRE11: meiotic recombination 11, MSH6: MutS homolog 6, MSH2: MutS homolog 2, MSI: MicroSatellite instability, NOTCH1: Neurogenic locus notch homolog protein 1, NRAS: Neuroblastoma RAS viral oncogene homolog, NTRK: Neurotrophic tyrosine receptor kinase, PIK3CA: Phosphatidylinositol-4,5-bisphosphate 3-kinase, POLE: DNA Polymerase Epsilon, Catalytic Subunit, PTEN: Phosphatase and TENsin homolog deleted on chromosome 10, RCC: Right-sided Colon Cancer, RNF43: Ring Finger Protein 43, SMAD2: Mothers against decapentaplegic homolog 2, TP53: Tumor protein P53*

consumption [5,6]. It is therefore necessary to study the molecular mechanisms of CRC carcinogenesis, as it can lead to the discovery of novel screening and therapeutic methods, which can assist the diagnosis and prognosis clinically.

Traditionally, the large bowel has been categorised into three main groups, the proximal or right colon, the distal or left colon and the rectum. The border of the right and left colon is the point between the proximal two thirds and the distal third of the transverse colon [7]. This reflects the differences in embryologic development [8]. From the midgut arise the superior mesenteric artery and vein, which vascularize the cecum, the ascending colon and the proximal two thirds of the transverse colon, while the distal transverse, the descending colon and the sigmoid colon are irrigated by the inferior mesenteric artery and vein, which derive from the hindgut [7]. Colon cancers are considered right sided or proximal if they are located before the splenic flexure. Left-sided colorectal cancers or distal carcinomas are cancers found distal to the splenic flexure. Tumours found in the splenic flexure are considered left-sided colon cancers [9]. Due to their embryologic origin, cancers of the right colon resemble gastric carcinomas and small bowel tumours [10,11]. It is of interest that neoplasms of the appendix and distal small bowel, although of shared embryologic descent and vascularisation with the right colon, are not included in the right sided colon cancer group [10]. They have differences in carcinogenesis and therefore are not in this group. Additionally, rectal carcinomas share similar molecular pathways with the distal large bowel and are considered left-sided cancers [11,12].

MOLECULAR BIOLOGY OF COLORECTAL CANCER

Multiple mutations of oncogenes and tumour suppressor genes occur in the oncogenesis process of colon cancer. Two main pathogenic pathways are involved in this sequence [13]. The first pathway involves the APC and β-catenin genes and features chromosomal instability. Normally, the APC tumour suppressor gene promotes β-catenin degradation [14]. The APC tumour suppressor gene is lost in this pathway, an event which promotes the development of an adenoma and occurs early in this process. The accumulation of β-catenin forces it to translocate to the nucleus. This activates the transcription of MYC and cyclin D1 genes. Mutations of the K-RAS gene begin to occur and subsequently mutations of the 18q21 and TP53 genes occur [13,15]. The second pathway consists of damage to DNA mismatch repair genes and accounts for approximately 10-15% of all cases of sporadic cancer [16,17]. The most common of these DNA mismatch repair genes to have genetic lesions is MLH1, resulting in a hypermutable state where repetitive DNA sequences, called microsatellites, become unstable during DNA replication [18]. This phenomenon, called microsatellite instability (MSI), characterises defective DNA mismatch repair, which results in the accumulation of mutations of growth-regulating genes and further development of colorectal cancer. In addition to these two main pathways, the CpG island methylator phenotype (CIMP) pathway is also involved in the CRC carcinogenesis [19].

Colorectal cancer is a heterogeneous disease which develops through various genetic and epigenetic mutations, with three distinct molecular pathways. These are chromosomal instability (CIN), microsatellite instability (MSI) and epigenetic methylation (Serated/CIMP) [19,20]. Chromosomal instability refers to many structural and numerical changes in chromosomes. This means whole chromosomes or parts of them are duplicated, inserted, or deleted, leading to aneuploidy [21].

Microsatellites, also known as Short Tandem Repeats (STR), are repeated sequences of DNA, with 1-4 bases per unit that are repeated and scattered throughout the genome, in areas that are coding or non-coding regions and account for about 3% of the entire genome [22]. Due to their repeated structure, they are susceptible to multiple errors and mutations during DNA replication. The system that corrects these errors is called DNA mismatch repair (MMR) [23]. Microsatellite instability is defined as the result of impaired MMR, which is phenotypically evident when there is a change in length of microsatellites. MSI occurs in genetically inherited mutations of MMR genes, such as Lynch syndrome, or in an epigenetic inactivation of these genes during methylation of MLH1 [18]. Carcinomas with high microsatellite instability are called MSI deficient, or MSI-d, whereas carcinomas with stable microsatellites are called MSI-proficient or MSI-p. Microsatellites that are unstable are highly immunogenic. This has an excellent effect with treatments of unstable tumours that activates the immune system [24,25].

GENES AND COLORECTAL TUMOR POSITION

Gene expression and tumor position

Gene expression in the normal colon varies between right and left side. For example, cytochrome p450 genes are expressed more in the right colon compared to the left colon in normal subjects. This may be due to differences in exposure of materials consumed in the colon [11]. Furthermore, methylation of genes is different on each side of the large bowel. The mismatch repair gene hMLH1 and

the O-6-methylguanine-DNA methyltransferase MGMT is found predominantly in the normal right colon of older females [11,26]. This may reflect epigenetic abnormalities that may lead to dysplasia and further development of adenocarcinomas of the right colon.

The CIMP phenotype consists of hypermethylation of CpG islands. These are clusters of cytosine-guanine complexes. CIMP is an epigenetic control aberration that is important for inactivation of onco-suppressor genes in cancer cells. Under normal circumstances, these areas are not methylated [20]. When hypermethylation occurs and onco-suppressor genes are inactivated, carcinogenesis may develop. According to the proportion of CpG islands methylated, tumours are divided into CIMP-high, CIMP low and CIMP-normal groups. CIMP-high tumours are often associated with microsatellite instability due to hypermethylation of MMR genes, and with BRAF mutations but are usually wild type for p53 mutations [27].

In 2015, in order to resolve inconsistencies in classifications of CRC based on gene expression, an international consensus decision was made on the molecular subtypes of colorectal cancer [28]. Four consensuses of molecular subtypes with distinguishing features were defined (CMSs). The CMS1 (microsatellite instability immune) subtype, consisting of 14% of CRCs, has the best prognosis but worse survival after recurrence. They are hypermutated and microsatellite unstable and are immunogenic; CMS1 samples were hypermutated and had low prevalence of somatic copy number alterations, and they had overexpression of proteins involved in DNA damage repair. As expected, the analysis of methylation profiles in TCGA showed that CMS1 tumours display a widespread hypermethylation status; The CMS2 subtype or canonical subtype is epithelial and occur in 37% of CRCs and have the highest overall survival. They detected more frequent copy number gains in oncogenes and copy number losses in tumor suppressor genes in CMS2 than in the other subtypes; the CMS3 subtype or the metabolic subtype occurs in 13% of cases and are also epithelial [29]. They also have an evident metabolic cancer phenotype; the CMS4 subtype, or the mesenchymal subtype occurs in 23 % of cases is prominent transforming growth factor-β activation, stromal invasion, and angiogenesis [30].

Molecular differences in colon cancer according to location can be seen using the molecular subtypes. In right colon cancer, CMS1 and CMS3 are more common, while CMS2 and CMS4 are more common in left colon cancer [11]. All subtypes are found on both sides, but the proportion is different according to location. CMS has been proved to be a significant clinical prognostic factor in overall survival (OR) and progression-free survival (PFS). In CMS1 groups, patients treated with bevacizumab had significantly better overall OS than those treated with cetuximab. In the CMS2 group, patients treated with cetuximab had significantly longer OS than patients treated with bevacizumab [31,32].

Carcinomas of the right colon are usually CIMP-high; they are also MSI high, hypermutated, and have a high affinity for BRAF mutations, especially the V600E mutation. Several other gene mutations such as KRAS, PIK3CA and RNF43 are found more frequently in RCC. Some gene mutations are exclusive to the right side. These genes are CDH1, MRE11, SMAD2 and NOTCH1 [11,33,34]. BRAF mutations and CIMP-high status have poor prognosis, giving right-sided colon cancer generally a worse prognosis than LCC [35,36]. Adversely, left sided colon cancers are microsatellite stable, they present chromosomal instability and APC and p53 mutations and genes that have Tyrosine Kinase Receptors are augmented, causing the upregulation of HER2 and EGFR genes [25,36,37].

Genes whose mutations are associated with cancer predisposing syndromes like Lynch syndrome, juvenile polyposis syndrome, PTEN hamartoma tumour syndrome, neurofibromatosis type 2 and hereditary breast ovarian cancer syndrome have slightly higher prevalence in RCC, which are namely the MSH6, MLH1, MSH2, POLE, PTEN, BMPR1A, BRCA1, BAP1, BRIP1, NF2, and MEN1 genes [11,26,37-39].

Differences in immunohistochemistry amongst right and left side cancers have also been identified. The expression of programmed cell death (PD-1) and PD-1 ligand-1 (PDL-1) is expressed approximately two times more in RCC rather than LCC [11,40,41]. The molecular differences mentioned are presented in Figure 1, Table 1.

THE ROLE OF THE MICROENVIRONMENT IN GENETIC DIFFERENCES

The microenvironment of the large bowel lumen plays an important role in the development of colorectal cancer. Environmental factors such as distinct microbiota, bile acid levels and chronic inflammation may contribute to carcinogenesis of the intestinal epithelial cells [44]. The microenvironment in the right side of the colon differs from the left side, which also affects the expression of genes between the regions.

Differences in the microbiota

The large bowel hosts a large number of different bacteria, including *E.coli*, and *F.nucleatum* and the percentage of these bacterial species is quite similar in the left side and the right side of the colon, accounting the microbe population of the colon as uniform [45]. Nevertheless,

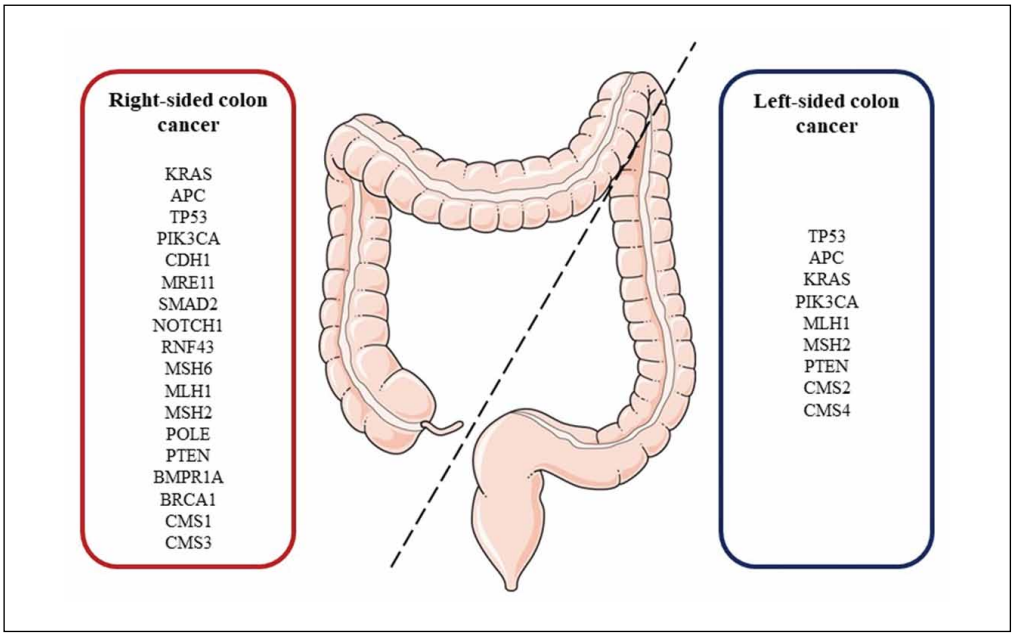

FIGURE 1. Main significant gene mutations in colon cancer per location.

this balance changes when colon cancer develops and differences in bacterial flora exist between patients with left and right sided colon cancer [46] .

Carcinogenesis related to bacterial exposure occurs via two different pathways. The first pathway has to do with chronic inflammation related to colitis and the bacteria responsible for this situation are usually *E. coli*, *B.fragilis, B.dorei, B.vulgatus* and *B.massiliensis* [47]. The second pathway consists of the creation of a microenvironment by different bacterial strains, which promotes immunological response and inflammation not related to colitis and bacterial strains involved in this second pathway are usually *F.nucleatum,* Pophyromonas, Parvimonas and Leptotrichiae [40].

E.coli are very common microorganisms and are part of the normal gut and the majority of group B2 *E.coli* can harbour genomic pks islands, which are responsible for the production of polyketide synthase [48]. In turn, polyketide synthase can cause double strand breaks in DNA which in turn causes an increase in γH2AX histones. These histones create polyploidy due to incomplete DNA repair, which can also create anaphasic bridges leading essentially to multiple mutations [49]. It is worth mentioning that the incidence of group B2 *E.coli* in biopsies of patients with right-sided colon cancer has been found to be higher in comparison to patients with left sided colon cancer [50].

F.nucleatum is the most studied of all oncogenic bacteria and has been found to have the most malignant potential and is found in a vast number of colorectal cancer patients, bound to mucin-producing cells of the intestinal lumen [51]. It binds to intestinal mucosa in two ways, with FadA and Fap 2 receptors. It promotes an

TABLE 1. Differences in characteristics of colon cancer according to location.

Right-sided colon cancer		Left-sided colon cancer	
Dominant characteristics	**References**	**Dominant characteristics**	**References**
More frequent mutations associated with cancer predisposing syndromes	[12,37,55]	Better prognosis and response to treatment	[31,32,53]
Higher incidence of group B2 E. coli	[50]	Microsatellite stability	[25,27]
Invasive biofilm	[47,57]	Chromosomal instability	[11,26]
Higher concentration of conjugated primary bile acids	[55]	Upregulation of Tyrosine Kinase Receptors	[36]

inflammatory microenvironment without colitis (NF-κB, IL-6,IL-8, IL-10, IL-18, and TNF) and via other pathways it creates an immune-deficient environment (by recruiting myeloid-derived suppressor cells or MDSC's with short chain fatty acids and polypeptides) by reducing CD3+ T-lymphocytes and promoting the beta-catenin pathway [52,53]. The result is cellular dysplasia and carcinogenesis. Interestingly, levels of *F.nucleatum* increase from rectum to cecum and accumulate gradually on normal colonic tissue in the adenoma-carcinoma sequence [53].

A layer of mucin containing bacteria on the luminal surface of the colonic epithelium is defined as bacterial biofilm [54]. This biofilm has been found to be invasive in approximately 90% of patients with colon cancer on the right side, while this has only been seen in about 10% of patients with cancer on the left side and subsequently, correlation between carcinogenesis and biofilms has been found in right colon cancer but not in left-sided cancer [55]. Epithelial E-cadherin has been found to be significantly decreased, whereas interleukin-6 is found increased and Stat-3 is activated when a biofilm is present, resulting in increased proliferation [56]. Association between high levels of pro-proliferative polyamine metabolite N, N-diacetylspermine, and biofilm in the lumen of the large bowel has been found, suggesting a relation between bacterial biofilms and host cancer [57]. Therefore, the formation of colonic bacterial biofilms with synchronous procarcinogenic epithelial responses has been suspected in the process of carcinogenesis in right-sided colon cancer [47].

Differences in bile acid levels

Bile acids (Bas) are produced in the liver by hepatocytes and only approximately 5%-10% of Bas pass the terminal ileum without getting absorbed and are deconjugated by bacterial bile salt hydrolases in the colon to secondary Bas [58]. Most of these molecules are absorbed by colon cells and returned to the liver to be reused. Bile acids, as well as their metabolites, have been associated with the development of colon cancer through different mechanisms such as angiogenesis, enhancing cancer cell proliferation, inhibiting apoptosis and assisting invasion [59]. The levels of these substances in the colonic lumen vary and are regulated by the normal colonic bacterial flora. Primary bile acids in the right colon interact with biofilms and microbiomes and are converted to secondary bile acids by means of deconjugation [60]. Deoxycholic acid (DCA) is the most found secondary bile acid and Lithocholic acid (LCA) is the second most common secondary bile acid. These acids are reabsorbed by the intestinal epithelial and subsequently alter DNA causing permanent damage through reactive oxygen and nitrogen species [59,60]. Conjugated primary bile acids are more commonly found in the right colon versus the left colon, almost 10 times more on the right [55]. Aspirates from the cecum and rectal fecal samples have been compared and have shown high levels of enzymatic activity, converting primary bile acids to DCA, in the cecal samples [61]. These findings suggest a possible role of differential bile acid levels according to location in colon cancer development.

Bas, when found in high concentrations in the large bowel, may cause cell membrane destruction, via their detergent properties, resulting in damage to intestinal epithelium. This situation promotes repair mechanisms that involve inflammatory cells and the proliferation and accumulation of undifferentiated cells [26,59]. This is a precancerous state which leads to formal carcinogenesis and CRC development. Furthermore, BAs have an oncogenic effect by making cells resistant to apoptosis [60]. This is possible by the degradation of tumor suppressor p53 by BAs which is responsible for cell processing of DNA repair and initiates apoptosis if DNA repair is not possible [62].

POLYPS IN COLORECTAL CANCER

Polyps are commonly found in the colon and are considered precursors of colonic adenocarcinomas. Tubular and tubulovillous polyps are seen both on the right as well as the left side of the colon but they may present high-grade dysplasia and evolve into cancer more often on the right side of the colon, especially when found in smaller sizes [27]. Sessile serrated adenomas are also predominantly found in the cecum, ascending and transverse colon [27,42]. Comparatively, sessile serrated adenomas, found in right-sided colon cancer, present CIMP high levels, MSI high levels, MLH1 methylation and BRAF mutation while this is not seen in conventional adenomas found on both right and left sided colon cancer, whereas the opposite is seen for CIN where this is present in conventional polyps and not in sessile serrated polyps [43].

CLINICAL IMPLICATIONS AND POTENTIAL THERAPEUTIC TARGETS

Colorectal cancer is a heterogeneous disease and is treated today based on the presence of MSI or driver mutations such as KRAS, NRAS and BRAF. Recent trials showed progress in the development of personalised treatments which use alternative genes. These alternative genes are possibly responsible for progression of disease. Alternative receptors tyrosine kinases beyond EGFR and HER2 and additional fusions beyond ALK and NTRK should be examined before initiation of treatment and can further improve outcomes in mCRC. Studies have

found the presence of certain mutations that may indicate contraindications for some treatments, such as ARHGEF33. This gene is similar to KRAS/NRAS activating mutations and has a negative impact in anti-EGFR treatment [63].

In addition, some targeted therapies that are already in use in other cancers with very good results, namely Sotorasib, harbouring the KRAS pG12C mutation in non-small-cell lung cancer, should be further investigated for potential use in CRC.

Presently several types of immunotherapies are applied in the treatment of CRC. These include monoclonal antibodies, ICB to reinvigorate T-cell immunity, CAR-T cell therapy, oncolytic viruses and cancer vaccines. Furthermore, the activation of the immune system with therapeutic DNA cancer vaccines is a very promising approach. Pre-clinical trials have shown that monotherapy with these vaccines have not changed the outcomes of cancer, but the combination with other personalized treatments based on the patient's genetic profile and biomarkers should be used. This way, effective treatments can be ensured and side-effects can be minimised [64].

CONCLUSIONS

As seen in this narrative, colorectal cancer presents major differences according to location regarding molecular characteristics which in turn, affects its histopathology, prognosis, and response to treatment. Overall, colorectal cancer cannot be considered a single disease but should be treated as 2 different diseases in the same organ [9,12]. The underlying causes of the reported molecular differences between colorectal tumor locations may be multifactorial. Environmental, genetic and immunological factors all play roles in the development and overall survival of colorectal cancer patients [26,32,40,53]. The clinical significance of these findings requires replication and additional studies need to be undertaken in larger populations. Indeed, nowadays, in the era of personalised medicine, sidedness is a major factor in the treatment of colorectal cancer and the biology and genetic pathways of this disease need to be studied further to determine potential targets for individualised treatment [63]. Therefore, there is a need for further research and broader genomic profiling for a better understanding of tumour biology, hopefully leading to new discoveries in diagnostics and therapeutics.

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Low-dose CT – *truly* helping a patient who won't quit smoking

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It is well past 7 pm and I have just finished the last case of the day in the operating room (OR). I head to the floors, where four patients are waiting for me to record their medical history and perform physical examinations and consent them for their upcoming surgeries. The first patient is a 68-year-old male with 90% stenosis of the left anterior descending artery, 80% stenosis of the circumflex, and total occlusion of the right coronary artery. He is scheduled for triple coronary artery bypass graft surgery. During our encounter, he reports smoking about 1.5 packs of cigarettes a day for over 30 years. When attempting to counsel him on the benefits of cutting back on his smoking, he quickly interrupts me and says "*Doc, thank you, but I'm way too worried about my upcoming heart surgery to even consider talking about smoking cessation*". The next patient is a 62-year-old diabetic female with severe aortic stenosis awaiting valve replacement surgery. Soon enough, the conversation came down to smoking. The patient knew all too well how detrimental smoking can be, especially to a diabetic. She tried quitting a few times in the past. The first time, she was really excited about leading a smoke-free life. A few weeks down the road, however, she got fired and relapsed. The second time around, she was going through a nerve-wrecking divorce and turned back to smoking as a way of releasing tension.

The third patient was a 35-year-old mother who had just got off the phone with her young daughter when I walked in the room. While interviewing her, she reported a 40-pack per year history of smoking. She was recently diagnosed with a large atrial septal defect and was utterly petrified. If anything, she had been smoking even more for the past couple of months. The fourth patient was a 60-year-old patient with chronic obstructive pulmonary disease undergoing right upper lobectomy for stage IIB adenocarcinoma who had repeatedly tried to quit smoking but failed despite giving it her best. She asked me whether there was anything she could have done that might have helped detect her cancer at an earlier stage. I could not help but wonder why her primary care provider (PCP) or pulmonologist never mentioned the option of low-dose chest CT (LDCT).

A few days later, I was seeing cardiac surgery patients for their one-month postoperative check. Although, upon discharge, all active smokers received counselling and were provided with resources to help them quit smoking, only one of them reported cutting down. Unfortunately, many people simply will not quit smoking despite their best efforts and the well-meaning guidance of their healthcare providers. Being fully aware of that harsh reality, should we re-evaluate our role as physicians in mitigating the catastrophic impact of smoking? Is it ethically acceptable to limit our efforts to just counselling against the use of tobacco products? Or should, for certain people, "*quit smoking*" be followed by "*get your annual CT*". If so, under which circumstances would such an intervention be best received by patients?

The answer is simple. The more the merrier. All encounters could be utilised to educate high-risk patients about this painless and potentially life-saving intervention. Universally, all physicians across all specialties, healthcare systems, and countries take several patient histories every day. During that interactive process, providers inquire about their patients' smoking history. When treating a heavy smoker, it is crucial not only to counsel them regarding potentially effective ways to curtail the use of

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tobacco products, but also to educate them about the value of screening.

The U.S. Preventive Services Task Force recommends yearly lung cancer screening with LDCT for people with a 20 pack-year or more smoking history, who are active smokers or have quit within the past 15 years, and are between 50 and 80 years old [1]. The CHEST/American Society of Clinical Oncology guidelines as well as the American Thoracic Society also support lung cancer screening with LDCT. It should be emphasised that LDCT has been shown to afford a 20% relative decrease in lung cancer death (from 1.66 to 1.33%, or 3 fewer deaths per 1,000 screened) as well as a 7% relative reduction in all-cause mortality [2,3].

To put these statistics into tangible perspective: The European Union currently has a population of approximately 447 million people of whom 30%, aka 134.1 million, are between 50 and 80 years of age. According to data from the European Commission, 6% (8,046,000) of those are heavy smokers [4]. If this high-risk subgroup of people underwent LDCTs annually that would lead to a staggering reduction in lung-cancer related deaths by 24,138 patients.

Despite these encouraging data, it has been shown that annual radiologic screening for high-risk patients is infrequently ordered by PCPs (0 tests: 33%, 1–5 tests: 36%, 6–10 tests: 16%, 11–24 tests: 10%, 25+ tests: 4%, not sure: 2%). Surprisingly, most PCPs who engage in lung cancer screening seem to rely on plain chest x-ray with less than half utilizing LDCT routinely [5].

It is high time for a paradigm shift. It does not matter if the provider is a medical student, a nurse, a gastroenterologist, a nephrologist, an ophthalmologist, a dermatologist, an orthopedist, or a neurosurgeon. Being proactive about eradicating smoke-related lung cancer is not, and should not be, solely the job of PCPs, pulmonologists, oncologists and thoracic surgeons. The infamous Swiss cheese model is the key. Truly, the more, the merrier.

So, do you have a patient that is eligible for LDCT? Let them know about this option. Many have never heard of it and those who have probably do not know a lot about it. Again, the truth of the matter is that many patients will never succeed in quitting smoking. But, if properly and routinely educated, they might consider getting an annual CT. It could save their life.

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Case Report

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A rare case of giant oesophageal liposarcoma treated with oesophagectomy

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ABSTRACT

Background: Oesophageal sarcomas are extremely rare, constituting less than 5% of oesophageal tumours, with only 0.5% (10%) of these being liposarcomas.

Aim: Presentation of a rare case of an oesophageal liposarcoma and review of the literature.

Case Presentation: A 69-year-old man with a three-year history of voice changes and progressive dysphagia was referred to our department for consultation and treatment of a giant dedifferentiated oesophageal liposarcoma. The patient underwent hybrid McKeown oesophagectomy.

Conclusions: Multimodal management of such patients in specialised centers may spare these patients the discomfort while offering the best chances for cure.

Key Words: *Liposarcoma; spindle cell; oesophagectomy*

INTRODUCTION

Sarcomas are a heterogeneous group of malignant tumours of mesenchymal origin that more commonly affect soft and bone tissue. Their incidence is approximately 5 cases per 100.000 population, with a slight male predominance [1,2]. Gastrointestinal tract sarcomas, in particular, are a rare entity, with a published annual incidence of 0.07 cases per 100.000 population [1]. Primary oesophageal sarcomas are even more infrequent, with the majority of oesophageal tumours arising from the mucosal lining and only 5% being from mesenchymal origin. Only 0.5% of these are liposarcomas, thus representing a challenging diagnosis [3].

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We present the case of a 69-year-old man diagnosed with a giant oesophageal liposarcoma, initially misdiagnosed and managed as a lipoma.

Case Presentation

A 69-year-old man of Caucasian descent was referred to our department for surgical management of a dedifferentiated oesophageal liposarcoma.

The patient had a three-year history of progressive voice change with a characteristic "hot potato voice" and worsening nocturnal breathing difficulty, with no associated odynophagia, dysphagia, lump sensation, heartburn or weight loss. The patient was assessed at an ENT department in March 2019, and endoscopy revealed a mobile mass obstructing two-thirds of the laryngeal inlet without involvement of the mucosa. The endoscopic excisional biopsy revealed a spindle cell lipoma of the larynx.

Fourteen months later, the patient presented with symptom recurrence. A flexible micro-laryngoscopy revealed a tumour originating from the internal laryngeal

wall, extending from the left aryepiglottic fold to the corresponding apioid fossa. The supraglottic part of the tumour was excised, and CO2 laser ablation of the residual lesion was applied. The patient had an uneventful postoperative period and was discharged on the 5th postoperative day.

Sixteen months later, the patient presented with dysphagia to solid food and weight loss. A repeat endoscopy revealed significant narrowing of the entire length of the oesophageal lumen (Figure 1). A chest CT scan showed a 18 cm long, fat-density lesion along the oesophageal wall. No mediastinal lymphadenopathy was found (Figure 2). The patient underwent endoscopic ultrasound (EUS) and fine-needle biopsy (FNB). The EUS reported a partially obstructive submucosal tumour of the oesophagus, extending from 20 cm to 38 cm from the incisors without extension to the other layers of the oesophagus. In addition, the submucosa of the stomach was found thickened up to 4 cm below the gastroesophageal junction.

The histological report revealed a mesenchymal tissue neoplasm consisting of pleomorphic spindle cells with high-grade nuclear atypia, suggestive of a dedifferentiated esophageal liposarcoma. Subsequent immunohistochemical analysis further supported this diagnosis: MDM2 (+), CDK4 (+), Vim (+), CkAE1/AE3 (-), S100 (-), DOG-1 (-), CD117 (-), SOX10 (-), CD45 (-), Desmin (-), Ki-67 25%.

Further imaging was applied for investigation of the mediastinal extent of the lesion. A chest MRI ascertained the lesion's intramural spread with no evidence of invasion to adjacent structures (Figure 3), while a PET/CT revealed a moderately hypermetabolic esophageal wall (SUV max=3,9) and esophagogastric junction (SUV max=7.3) with no findings of distant metastases.

Surgical Management

A three-field hybrid McKeown oesophagectomy was performed. The resection consisted of a right thoracoscopy, a midline laparotomy and a left cervical incision (Figure 4). A hand-sewn single-layer end-to-side oesophagogastric anastomosis of the cervical esophagus to the gastric conduit was performed in the neck after radical resection. A feeding jejunostomy was also placed. An 18Fr chest tube was placed on each pleural space. The overall duration of

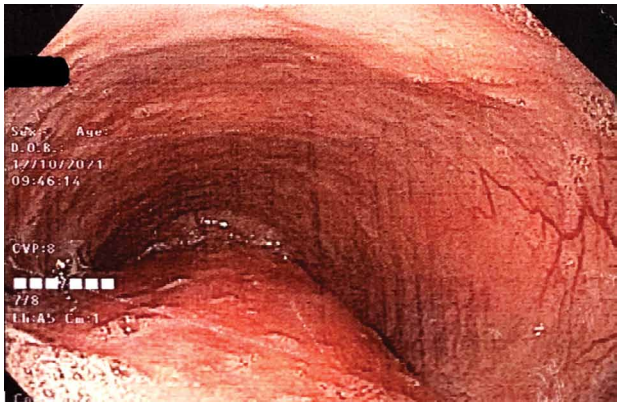

FIGURE 1. Endoscopic image showing the narrowed oesophageal lumen, with no signs of mucosal infiltration.

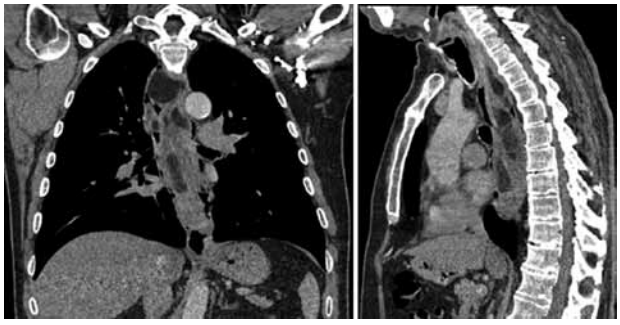

FIGURE 2. Coronal (a) and sagittal (b) chest CT images showing an 18cm long, fat-density lesion along the oesophageal wall. Neither direct invasion to adjacent structures nor mediastinal lymphadenopathy was noted.

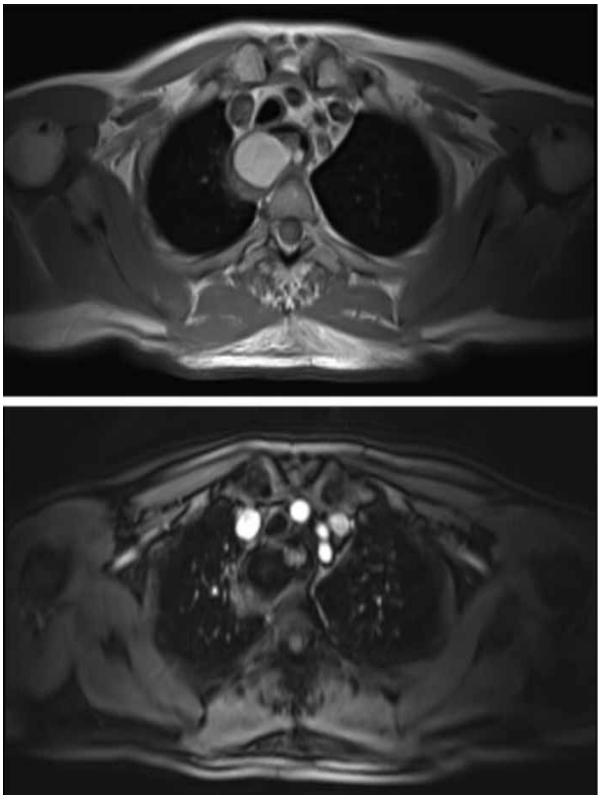

FIGURE 3. T1W (a) and T1W FS (b) chest MRI images showing the predominantly fatty composition of the intramurally spread of the oesophageal lesion. No evidence of invasion to adjacent structures is shown.

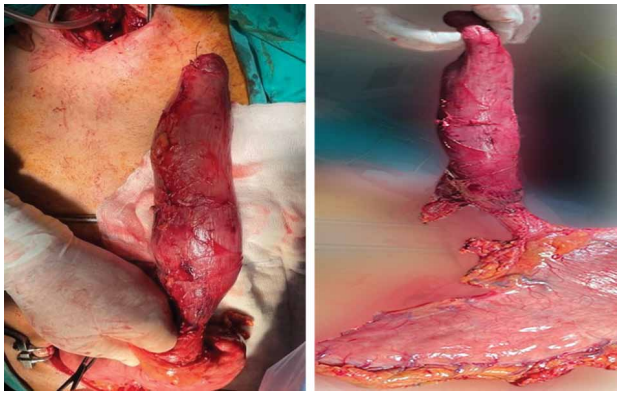

FIGURE 4. (a,b) Intraoperative images showing the dissected oesophagus, with prominent distention above the stenotic lesion.

the procedure was 5 hours and 45 minutes. The procedure was well tolerated without intraoperative complications and with minimal blood loss. The patient was successfully extubated and was admitted to the ICU for early postoperative monitoring during the 1st postoperative day (POD). On POD 2, the left chest tube was removed as there were no signs of pleural effusion or pneumothorax, and the patient was started on enteral feeding via the jejunostomy. On POD 4, oral feeding was initiated and the right chest tube was removed. Two days later, the patient presented mild swelling and redness in the cervical incision and an anastomotic leak was confirmed and drained bedside; a course of intravenous antibiotics was administered for 5 days and oral feeding was resumed during the second postoperative week. The patient remained hemodynamically stable with no systemic inflammatory signs during the hospital stay and was discharged on POD 14 on enteral feeding via the feeding jejunostomy tube.

Specimen's Histopathology Report

On macroscopic assessment, the length of the esophagus was 21cm. A 19x6x4 cm polypoid mass was developed intramurally. The microscopic examination revealed the presence of pleomorphic adipocytes with nuclear atypia and associated stromal cell atypia, and variable numbers of lipoblasts. The immunohistochemically analysis, was positive for MDM2, CDK4 and CD34, Ki67 expression was 30% (Figure 5). Retrieved lymph nodes had no signs of malignant infiltration.

Literature review

Materials and Methods

A search in PubMed was conducted aiming to identify cases of esophageal liposarcoma. Literature reviews, case

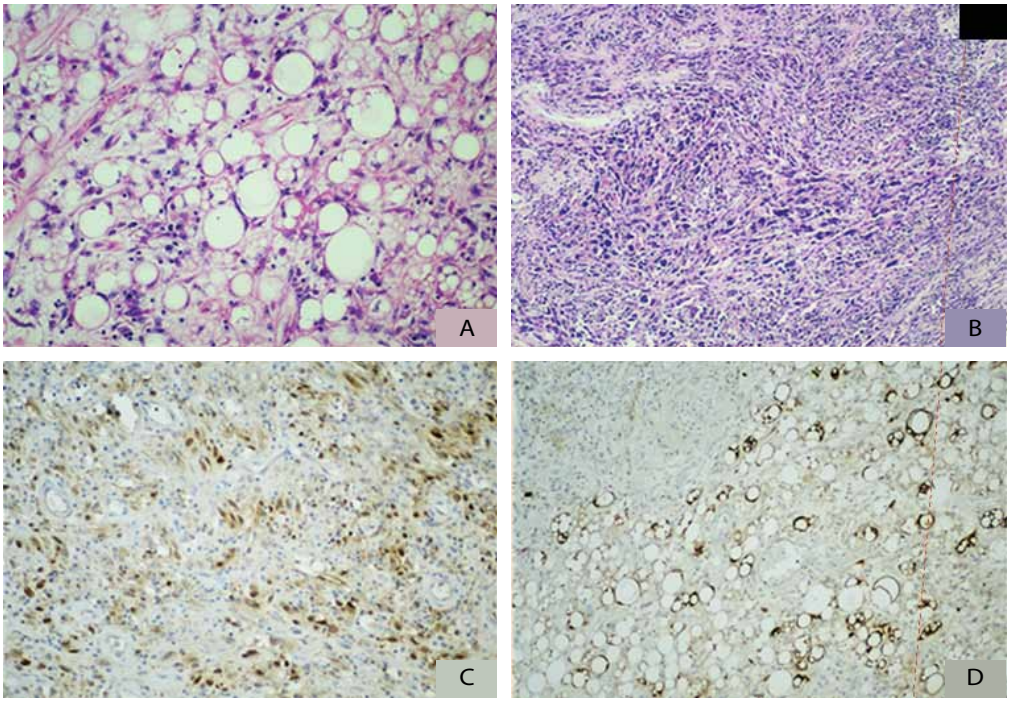

FIGURE 5. (a): Histological image of well-differentiated liposarcoma. Note the abundant lipoblasts, with hyperchromatic nuclei and lipid-rich droplets in the cytoplasm (Hematoxylin-Eosin x40). (b): Histological image of a dedifferentiated liposarcoma, composed of highly atypical cells (Hematoxylin-Eosin x20). (c): CDK4 immunohistochemical staining shows positive expression of the tumor. (d): S100 immunohistochemical staining highlights lipoblasts.

reports and one systematic review were also included in our analysis. Only English-language articles were included.

RESULTS

A total of 15 cases of esophageal liposarcoma ,including our case , were identified (Table 1) [4-17]. The mean patient age was 58.7 (42 - 83) years. 3 of the patients were female (20.0%) of the patients One patient was diagnosed with liposarcoma of the gastroesophageal junction; therefore, excision of the distal esophagus and a total gastrectomy were performed [17].

Progressive dysphagia was the most common complaint, being present in 13 out of 15 patients (86.6%). Other common symptoms were weight loss (5 patients, 33.3%) and dyspnea (4 patients, 26.6%). In addition, 2 patients (13.3%) complained of either nausea, chest discomfort, vomiting, or voice change. Less common symptoms included anorexia, palpitations, throat discomfort, foreign body sensation, night sweats, cough, and retrosternal pain.

The duration of symptoms before treatment varied significantly among the patients, ranging from 1 month to 17 years [11,17]. This variation is explained by the fact that many patients had undergone other less invasive treatments prior to esophagectomy, and the decision for radical resection was taken to achieve complete resection after local recurrence.

Regarding the tumours' location, the majority extended from the cervical part of the esophagus towards the middle or even the distal part of the organ. Interestingly, most patients had either a dedifferentiated liposarcoma or a well-differentiated liposarcoma with a dedifferentiated component (6 patients, 40.0%) [6,9,12,13,17].

Following the operation, 2 patients (13.3%), including ours, developed an anastomotic leakage, and one of them required a reoperation [4]. Moreover, two patients developed a benign anastomotic stricture 7 and 12 months after esophagectomy, with both being treated with endoscopic dilatations [10,11]. No deaths occurred in the early postoperative period.

DISCUSSION

Soft-tissue sarcomas (STS) are rare tumours accounting for 1% of all adult malignancies, occurring predominantly in the trunk, the extremities, and the retroperitoneum. They comprise more than 100 histologic subtypes. The most common subtypes are the liposarcoma, the leiomyosarcoma, and the undifferentiated pleomorphic sarcoma [18]. The 2020 WHO Classification of Soft Tissue Tumours classified the malignant adipocytic tumours into five categories: well-differentiated liposarcoma, dedifferentiated liposarcoma, myxoid liposarcoma, pleomorphic liposarcoma and myxoid pleomorphic liposarcoma [19].

Liposarcoma of the esophagus is extremely rare. Since the first report by Mansour in 1983 [20], less than 70 reports have been published in the literature, with the largest series of 13 patients coming from Graham et al [21]. In 2020, Ferrari et al. [22] published a systematic review about esophageal lipomatous tumours, including 65 case reports and two reviews. The total number of patients was 239, including 176 diagnosed with lipoma and 63 with liposarcoma. The median age was 66 years for the latter patient group, and the majority were men (73%). Furthermore, most of the tumours (73.0%) were found in the cervical part of the esophagus and protruded intraluminally (85.7%). Only 6 out of the 63 patients had a dedifferentiated liposarcoma on histology.

Ferrari also proposed an algorithm for the diagnosis, treatment, histopathological assessment, and surveillance of esophageal lipomatous tumours. According to this, an intraluminal lipoma that is larger than 15cm should be excised, either through a left cervicotomy or abdominal esophagectomy. In case that routine histology and MDM2 amplification on FISH confirm the diagnosis of liposarcoma, evaluation of the resection margins will guide the necessity for further excision or surveillance with CT imaging and endoscopy. We applied this algorithm to our patient, though already been subjected to two incomplete resections in the previous two years.

The mainstay of treatment is clear margin resection. This being said, several endoscopic or surgical techniques have been developed. Endoscopic techniques entail the endoscopic placement of a retraction suture followed by division of the tumour's stalk using either knife, ultrasonic shears, or electrosurgery snare, with or without hemoclip placement [23]. In cases where endoscopic resection is not feasible, surgery would be the standard resection mode. Surgical resection and reconstruction may include esophagostomy, esophagectomy and laparotomy for resection and retrieval of the tumour 23. In a systematic review by Dowli et al, including 40 cases of esophageal liposarcomas, the main reason for esophagectomy was the presence of a large, sessile submucosal tumor in need of clear resection margins [3].

Our patient was referred to our department after a histological diagnosis of esophageal liposarcoma was attained.

Here, the initial diagnosis two years before esophagectomy was spindle-cell lipoma, a benign lipomatous tumour, whereas the definitive histological diagnosis of the surgical specimen was WDLPS with a dedifferentiated component. Dedifferentiated liposarcoma (DDLPS) can

TABLE 1. Demographics, clinical presentation, histology, treatment immunohistochemistry and complication in 15 cases treated with oesophagectomy.

Author	Age	Gender	Presenting Symptom	Duration of symptoms until esophagectomy	Histology	Treatment	Type of lesion	Dimension (cm)	Location	Immunohistochemistry	Complications
Vouros (Greece)	69	M	voice change, dyspnoea, Dysphagia (recurrence)	34 months	Well-differentiated with dedifferentiated component	Mc Keown oesophagectomy (recurrence)	Polypoid mass	19x6x4	Upper, middle	CDK4(+) CD34(+) S100p(+) MDM2(-) SMA(-) Ki67=30%	Anastomotic Leakage/ Conservative management
Bak[4] (S. Korea)	49	F	Dysphagia, weight loss, anorexia, nausea, palpitation, chest discomfort	3 years	Well- differentiated	Total oesophagectomy	Polypoid mass	20x7	Upper, middle, lower	NA	Anastomotic Leakage/ Reoperation 8th POD
Chung [5](S. Korea)	56	M	Dysphagia, voice change, throat discomfort, foreign body sensation	13 months	Liposarcoma	Total laryngo-pharyngo-oesophagectomy	Polypoid mass	21x6x2	Upper, middle	NA	NA
Czekajska-Chehab [6] (Poland)	56	F	Dyspnoea	9 months	Well-differentiated with dedifferentiated component	Thoracotomy/ oesophagectomy	Polypoid mass	21x18x15 (Recurrence)	Upper, middle	NA	NA
Garcia [7] (USA)	42	M	Dysphagia, weight loss, vomiting, nausea, bleeding	3 months	pleomorphic	Transhiatal total oesophagectomy	Ulcerated, friable transmural mass	10,5x7x5,5	lower	S100 (+) in the better-defined lipoblasts. C-Kit(-), HMB-45(-), Melan A(-) and desmin (-) The more pleomorphic areas: CD68(+) and showed rare cytokeratin-positive cells.	Pulmonary oedema, bilateral pleural effusions, and pneumonia secondary to Klebsiella pneumoniae and Candida albicans.
Nakazawa [8] (Japan)	83	M	Chest Discomfort, vomiting	NA	Liposarcoma	partial oesophagectomy	Submucosal	12	middle	spindle cells: CD34(+) Adipocytes and lipoblasts: S100(+)	NA
Watkin [9] (France)	50	M	Dysphagia, weight loss, dyspnoea, night sweats, cough	NA	Dedifferentiated	subtotal oesogastrectomy	Submucosal lesion with exophytic component	10x8x6	lower	AE1/AE3 (-), CD34(-), desmin(-), S100(-), CD117(-), ALK1(-), CD21(-), CD23(-), CD35(-), CD30(-), EMA(-), MDM2(+), CDK4(+) (PCR), MDM2 amplification (FISH)	NA

TABLE 1. Demographics, clinical presentation, histology, treatment immunohistochemistry and complication in 15 cases treated with oesophagectomy *(continued)*.

Author	Age	Gender	Presenting Symptom	Duration of symptoms until esophagectomy	Histology	Treatment	Type of lesion	Dimension (cm)	Location	Immunohistochemistry	Complications
Yates [10] (U.K.)	49	M	Dysphagia, retro-sternal pain, weight loss	6 1/2 years	Myxoid	oesophagectomy (recurrence)	polypoid, intraluminal mass	NA	upper, middle, lower	NA	mild anastomotic stricture-dilations 7 months postop
Cooper [11] (U.K.)	68	M	Dysphagia	1 month	Myxoid	subtotal oesophagectomy	intraluminal, polypoid mass	7	lower	NA	benign anastomotic stricture 1 year postop
Lin [12] (China)	51	M	Dysphagia	6 months	Well-differentiated with dedifferentiated component	Mc Keown oesophagectomy	Submucosal	14x7x6,5	upper, middle	Vimentin (+), S100(+), CD34(+), CD117(-), CDK4(+), MDM2(+) (weak expression)	Uncomplicated
McCarthy [13](Canada)	61	M	Dysphagia	3 months	Well-differentiated liposarcoma (ALT/ WDL), with a focus of low-grade dedifferentiation.	Mc Keown oesophagectomy	Polypoid	21	upper, middle, lower	MDM2 (+) (FISH), CD34(-), S100(-), SMA (-)	Uncomplicated
Mehdorn [14] (Germany)	75	M	Dysphagia, weight loss	NA	Well- differentiated	Thoracotomy, oesophagectomy	Polypoid	25x10	upper, middle	MDM2 (+) (FISH), CD34(+), S100(+), SMA(+)	Uncomplicated
Sui [15] (China)	49	F	Dysphagia	3 years	Well- differentiated	Subtotal ooesophagectomy	Transmural ellipsoid mass	12x6x4	middle, lower	NA	Uncomplicated
Rui Li [16] (China)	52	M	Dysphagia, dyspnoea	NA	Liposarcoma	Laparoscopic and thoracoscopic oesophagectomy	Polypoid mass	21x5,1 cm	upper, middle	adipose cells: CDK4(+), S100 (+) Discrete spindle cells: Desmin (+), CD34(+), S100(-), DOG1(-), SMA(-), STAT(-)	Uncomplicated
Askan [17] (Turkey)	71	M	Dysphagia	17 years (reccurence X2)	Dedifferentiated	Total gastrectomy with distal oesophagus	solid mass protruding into the lumen	3X2,5X11,1	lower (GEJ)	MDM2 (+) (FISH), CDK4 (+), CD34(-), CD117(-), DOG1(-), SMA(-), S100(-), pan cytokeratin(-), desmin (-), HMB45 (-), SOX10(-)	NA
Sum	58,7	M:12, F;3									

M: male, F: female, NA: not applicable, POD: postoperative day, SMA: smooth muscle actin

either occur de novo (90%) or during a recurrence of a preexisting well-differentiated liposarcoma (10%) [17]. If this is the case, dedifferentiation of WDLPS will occur at 20% during the first recurrence and 44% during the second recurrence [17].

In addition, it is relatively common for an oesophageal liposarcoma to be initially misdiagnosed as a "giant fibrovascular polyp" or a "lipoma." Graham et al. reexamined the clinicopathologic features and MDM2 amplification status of 13 cases initially diagnosed as: "lipoma" (n=1), "giant fibrovascular polyp" (n=5), "WDLPS" (n=3) and "DDLPS" (n=3)4. Interestingly, a woman with a 7cm tumour initially diagnosed as a lipoma exhibited MDM2 amplification and was finally identified as a well-differentiated liposarcoma. In the same study, five patients with "giant fibrovascular polyp" had differentiated liposarcoma as their final diagnosis [21].

Although surgical resection is the cornerstone in the treatment of esophageal liposarcoma, a close long-term follow-up is strongly recommended since approximately 40% of DDLPS tend to recur locally and 17% tend to metastasize distantly. Eventually, 28% of the patients die as a result of distant spread [24]. Our patient remains disease-free, with no signs of local or distant recurrence, five months following esophagectomy.

CONCLUSION

The present study presents the unusual case of an oesophageal liposarcoma initially misdiagnosed and treated as a benign lipomatous tumour. A significant difficulty in diagnosing such lesions stems from its rarity, with less than 70 cases published in the international literature. Furthermore, accurate assessment of subtle histological characteristics and specific immunohistochemical testing are mandatory, , whereas multimodal assessment in specialized centers and meticulous assessment through endoscopic and imaging studies might improve the overall prognosis of this rare entity.

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Ethical Standards: *1) This case report has been approved by the hospital's ethics committee (Hippokrateion General Hospital) and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. 2) All persons gave their informed consent prior to their inclusion in the study.*

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Case Report

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Retroperitoneal mass revealed to be metastatic lymph node of unknown primary origin

Cancer of unknown origin

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ABSTRACT

Cancer of unknown primary origin is a rare form of metastatic solid tumour representing less than 2% of malignancies. These patients tend to have an unfavourable prognosis, with long-term survivors scarcely reported in the literature. Here we present a rare case of a 56- year-old female with eight year survival, after surgical resection of a metastatic retroperitoneal lymph node, despite relapse of her tumour and no adjuvant treatment. We hope that this case report will aid in increasing awareness and understanding of this often overlooked entity.

Key Words: *Cancer of unknown primary; cancer of unknown origin; CUP, retroperitoneal mass; case report*

CASE REPORT

A 56-year-old female was referred to the surgical outpatient service due to an intraabdominal mass noted as an incidental finding in an abdominal computed tomography (CT). The patient was asymptomatic and had a normal physical examination at the time of referral apart from some vague abdominal pain. She had a past medical history of Hashimoto's thyroiditis, two unsuccessful in vitro fertilisation procedures and reported a family history of urinary bladder carcinoma on her father's side.

Her abdominal CT scan and magnetic resonance imaging (MRI) depicted a 6-cm, well-circumscribed solid mass, with heterogeneous enhancement. The mass was located in the retroperitoneum between the head of the pancreas and the great vessels and displaced the inferior vena cava and the third part of duodenum. Differential diagnosis included gastrointestinal stromal tumour (GIST), neurogenic tumour, other tumour of mesenchymal origin or metastatic lymph node (Figure 1).

Since a metastatic lymph node was suspected, a complete workup was scheduled to identify the primary tumour including tumour markers, endoscopy of the upper and lower gastrointestinal tract, chest CT, mammography, cervical smear, transvaginal ultrasound and head MRI without any remarkable findings. In order to establish a conclusive diagnosis, a CT-guided biopsy of the lesion was performed. The results of the histopathological examination showed poorly differentiated adenocarcinoma of unknown origin.

Subsequently, the patient was hospitalised to undergo surgical resection (Performance Status 0). During surgical exploration, two well-circumscribed scleroelastic lesions were identified in the retroperitoneum in close proximity to the great vessels, the right ureter and the lower pole of

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ABBREVIATIONS: *CA-125: cancer antigen 125, CT: computed tomography, CUP: cancer of unknown primary, GIST: gastrointestinal stromal tumor, LDH: lactic dehydrogenase, MRI: magnetic resonance imaging*

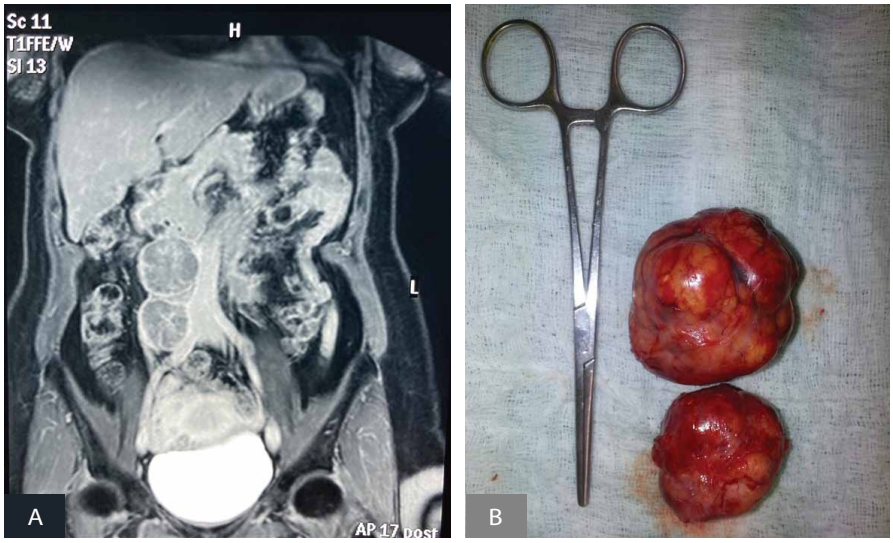

FIGURE 1. (A) Preoperative abdominal MRI depicting a bilobular well circumscribed mass with heterogeneous enhancement in close relation to the great vessels. (B) Surgical specimen after resection. A Kocher forceps is shown, for size reference.

the right kidney, which were resected en bloc. Her postoperative course was uneventful and she was discharged from the hospital on the 7th postoperative day. Histopathological examination reported a metastatic lymph node due to poorly differentiated carcinoma of possible primary pancreatic, breast or ovarian origin. However, as mentioned above, no primary tumour was discovered in these or any other possible location.

The patient denied any further treatment. Two months after surgery, the MRI showed residual tissue at the resection site with a largest diameter of 1,5cm. Her annual follow-up with MRI and blood workup showed no evidence of recurrence. Four years after surgery, cancer antigen 125 (CA-125) level increased to 580 U/ml in parallel with an increase in the diameter of the residual lymphatic tissue to 2.7cm and the appearance of a second enlarged lymph node near the right common iliac artery. The lymph nodes were hypermetabolic in positron emission computed tomography (PET-CT) scanning, while no other lesions were identified. The following years a gradual increase in the CA-125 level and in the diameter of the residual lymphatic tissue was noticed, being 1930 U/ml and 6cm respectively, at her eight year follow up (Figure 2).

Remarkably, the patient continues to remain asymptomatic even though she has denied conservative treatment or surgical resection over these years.

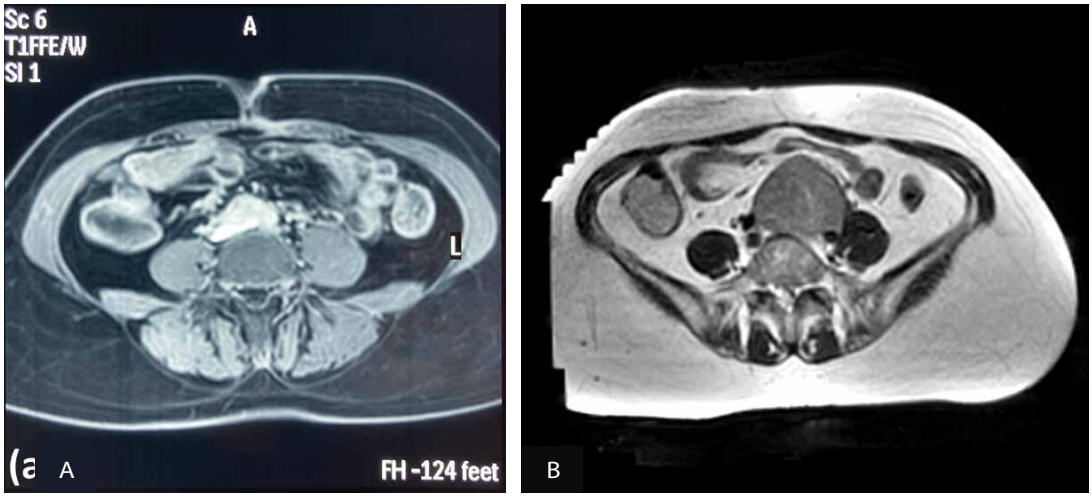

FIGURE 2. (A) Abdominal MRI two months postoperatively, showing residual tissue near the aortic bifurcation. (B) At her last follow-up, abdominal MRI indicates relapse of her tumour.

DISCUSSION

Cancer of unknown primary (CUP) is defined as a metastatic solid tumour, in the absence of a primary cancer after complete diagnostic work up of the patient. [1] This definition excludes patients with a metastatic tumor as the first manifestation, for which the primary tumor was eventually revealed in the diagnostic process. The incidence of CUP has decreased in the course of time, as diagnostic tools have emerged to assist in identifying the primary site, and is currently estimated to be less than 2%. [2] Nevertheless, there is a percentage of patients for whom the primary remains elusive even at autopsy.

Four main histological subtypes of CUP have been described. These include adenocarcinoma (50%), undifferentiated carcinoma (30%), squamous-cell carcinoma (15%) and undifferentiated neoplasms (5%), which are then further subcategorised, after thorough investigation with immunohistochemistry markers [3]. If the origin cannot be identified, genetic assays have recently been used to guide therapeutic decisions [4]. In this case, even after a thorough immunohistochemistry investigation by two independent pathology laboratories and complete diagnostic work up, no primary was identified. She therefore fulfills the criteria for the diagnosis of CUP, specifically CUP of midline distribution.

There is no consensus regarding treatment of CUP according to the published literature. For the majority of patients with CUP that don't belong to a specific subgroup, morbidity and quality of life play an important role in decision-making. Patients with midline distribution CUP show better response rates, as well as better outcomes after platinum-based chemotherapy [5]. Therefore, the appropriate management for this case should include resection with clear margins and adjuvant chemotherapy with a platinum based agent - she was treated by surgical resection, however she refused to receive any adjuvant treatment.

CUP phenotypes with a more favorable prognosis, include women with adenocarcinoma of the axillary lymph nodes, squamous carcinoma involving cervical lymph nodes, CUP with neuroendocrine features, CUP of a single location, men with midline CUP and women with peritoneal papillary serous carcinoma [1].

Several prognostic systems have been developed to predict the survival of CUP patents, irrespective of the specific subtype, including Culine's prognostic score [6], which is the most widely accepted, validated model based on Performance Status and lactate dehydrogenase (LDH) levels (predicted median survival 11,7 months in the favorable prognosis group). Pentheroudakis et al (2010) [5] suggested low tumour bulk, patient fitness, female gender, carcinomatous histology, and absence of visceral metastases as positive predictive markers specifically in CUP of midline distribution, with survival barely touching the one-year mark.

At her six-year follow-up, despite no adjuvant therapy and a relapse of her tumour, our patient is still asymptomatic and in impeccable general condition. Although the favourable outcome is in line with the lack of adverse prognostic indicators (poor Performance Status, liver metastasis, elevated LDH) discussed above, it still surpasses even the most optimistic prognostications.

Even though there have been scarce reports of long-term survivors in the literature [7], this is the only reported case of CUP, to our knowledge, with a long-term survival despite relapse that received no systemic treatment. This case therefore questions our current understanding of CUP, including the overall benefit of adjuvant chemotherapy in prolonging survival and raises the question of the necessity of surgical resection in the first place.

Ethical standards declaration: *This work complies with the current Greek law requirements. No ethics committee approval was required for the case described in this manuscript.*

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Median Arcuate Ligament Syndrome: A Case Report

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ABSTRACT

Median Arcuate Ligament Syndrome (MALS) is a rare vascular compression disorder of the celiac artery with non-specific symptomatology that can mimic other, common abdominal diseases. We present one such case of MALS and review the relevant literature on this rare topic.

Key Words: *Artery; compression; celiac; diaphragm; fundoplication*

INTRODUCTION

The Median Arcuate Ligament (MAL) is an arch-like fibro-fascial ligament that is formed by the fibres of the right and left diaphragmatic crura along the anterior border of the aortic hiatus [1-3]. Anatomical variations leading to superior origin of the celiac trunk from the aorta and low insertion of MAL over the origin of the celiac trunk may cause compression; when compression is significant, it can present with a wide variety of symptoms that may range from chronic epigastric pain, nausea, vomiting, epigastric fullness and delayed gastric emptying [1-4]. This rare condition is known as the Median Arcuate Ligament Syndrome (MALS), Dunbar syndrome, or even, celiac artery compression syndrome [1-4].

CASE REPORT

A 27-year-old male presented in the emergency with symptoms of dull upper abdominal pain, anorexia and weight loss for a period of two years. He had been diagnosed as a case of hiatal hernia earlier and had undergone Nissen fundoplication nine months ago, but his symptoms had not settled after surgery. On physical examination the abdomen was soft and non tender and his laboratory studies were within normal limits. Ultrasound of the abdomen was non-contributory. A contrast enhanced computed tomography (CECT) scan of the abdomen was performed, which showed narrowing of the celiac trunk at its origin, measuring approximately 1 mm in transverse axis with post stenotic dilatation with thickened median arcuate ligament, suggestive of median arcuate ligament syndrome. Computed tomography angiography (CTA) revealed an attenuated celiac artery at its origin along with post stenotic dilatation (Figure 1).

The patient was taken up for surgery. Intra-operatively, the right and left diaphragmatic crura were identified and the MAL was seen covering the origin of the celiac trunk off the aorta; the celiac artery was thread-like, with faint pulsations. The MAL was divided in the midline and all soft tissue including lymphatics were carefully dissected to widely expose the aorta and celiac trunk and a clear view of the anatomy was obtained (Figure 2). Once this was done, the celiac artery showed an immediate increase in caliber and pulsations.

The post-operative course was uneventful and the patient was discharged on the second postoperative day without any dietary restriction.

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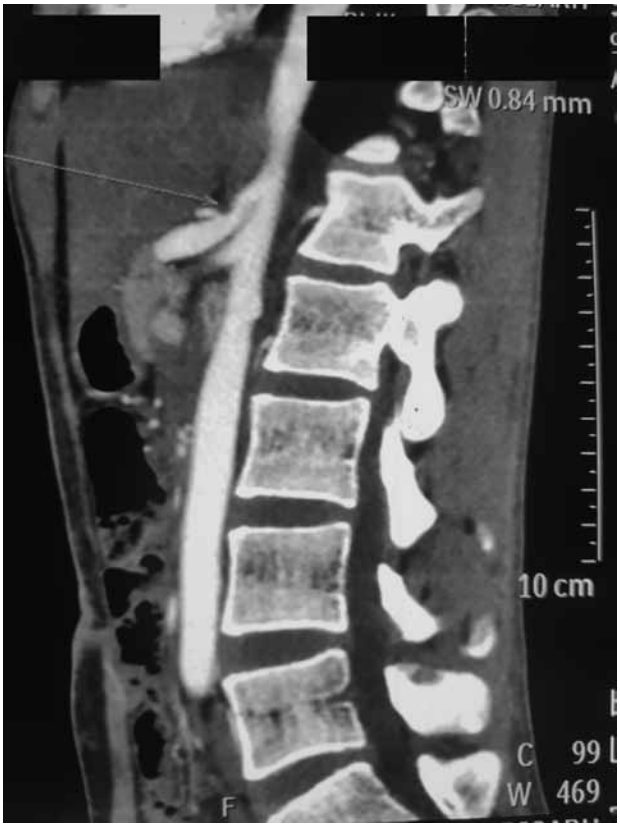

FIGURE 1

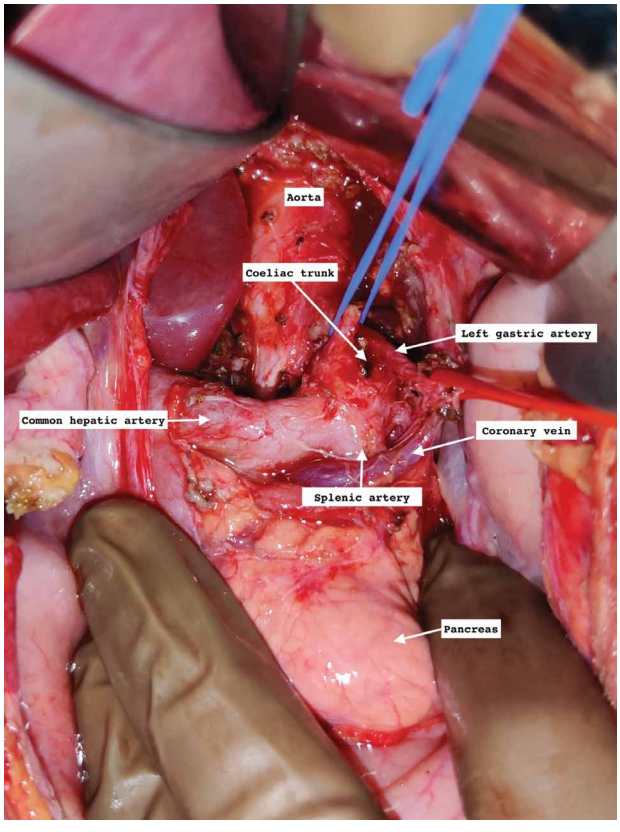

FIGURE 2

DISCUSSION

Ever since the initial descriptions by Harjola (1963) and later by Dunbar (1965), MALS has remained a rare diagnosis, usually by exclusion of other causes [1,3]. It is seen more commonly in thin females in the 3rd to 5th decades of life and presents with chronic epigastric pain (post-prandial or triggered by exercise), nausea, vomiting, weight loss and bloating [1-5]. The exact pathophysiology of MALS is still unclear - most current theories revolve around gastrointestinal ischaemia arising from mechanical compression of the celiac artery by MAL; however, compression of the celiac nerve plexus by MAL may also contribute [1,4]. Usually, the MAL transverses the aorta cranial to the origin of the celiac artery, but low insertion of MAL has been reported in up to 24% of the population [1]. In addition, there may be anomalous higher origin of the celiac trunk from the aorta, and any of these, alone or in combination, may cause compression of the proximal celiac artery, especially during expiration [1,3,6]. However, simply, compression does not always translate into MALS; compression of the celiac artery on angiography is seen in almost 50% of individuals, but not all are symptomatic [6,7].

Clinically, MALS can range from being completely asymptomatic to having very general presenting symptoms. The typical symptoms reported in MALS are post-prandial abdominal pain, weight loss, nausea and vomiting; other presentations include anorexia, nausea, vomiting, fatigue and diarrhea, similar to several other abdominal disorders like gastritis, peptic ulcer disease, hepatitis, cholecystitis, chronic pancreatitis, colorectal malignancy, appendicitis or chronic mesenteric ischaemia [1-7]. On occasion, abdominal bruit (increasing during expiration) may be heard. Given the rarity of the disease and its non-specific symptomatology, the diagnosis is difficult, and depends upon specific findings on abdominal Doppler, CT or MR angiography [1,4-7]. Abdominal doppler is a good initial investigation as it is easy to perform, avoids radiation and contrast, and can reveal post stenotic dilatation and increased flow velocities in the celiac artery that normalise on inspiration [1,2,5]. A combination of a deflection angle of greater than 50% and a high expiratory peak systolic flow velocity (more than 350 cms/sec) is highly sensitive and specific for the diagnosis of MALS [6]. However, this investigation is highly operator dependent, as well as limited by overlying bowel gas. Although conventional angiography is considered the 'gold standard' for diagnosing MALS [1], CTA has the advantage of high resolution three-dimensional reconstruction of the celiac axis as well as better visualisation of any other abdominal pathology [1,5-7]. Focal narrowing of the proximal celiac

artery and the presence of 'J-hook' pattern that normalises on expiration is considered diagnostic; in addition, CTA has better pick up of post-stenotic dilatation, distal aneurysms and collaterals [5-7]. MR angiography can be used instead of CTA in patients with contrast hypersensitivity or renal failure [1].

Surgery remains the mainstay of treatment [2]; decompression of the celiac artery by division of MAL and celiac ganglionectomy is recommended in symptomatic patients having documented compression on imaging in inspiration [1,2,6]. This can be done by open, laparoscopic or robotic means, although there is a recent trend towards the laparoscopic approach [4,7]. It has been reported that up to 85% of patients have symptom relief in the immediate post-operative period; typical pain (post-prandial or exercise induced), age between 40 to 60 years and weight loss greater than 10 kg are predictive of success after surgery [4,6]. Patients with persistence or recurrence of symptoms in the long term need evaluation for persistent stenosis/restenosis of the celiac artery either due to pathological changes (intimal fibrosis, smooth muscle proliferation, abnormal elastic fibres, disruption of medial and adventitial layers) that is often seen in long standing MALS, or abnormal anatomical configuration [4]. The treatment of such patients is by percutaneous transluminal angioplasty (with or without stenting) or arterial reconstruction [1,2,4].

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Disseminated ovarian alveolar rhabdomyosarcoma as a rare cause of peritoneal carcinomatosis

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ABSTRACT

Peritoneal carcinomatosis due to the presence of primary ovarian rhabdomyosarcoma of alveolar subtype (OARMS) is an extremely rare malignant tumour, with very few cases reported in the international literature. Herein, we present an interesting case of diffuse peritoneal dissemination of OARMS, in a previously asymptomatic patient, who was investigated for progressive abdominal distension.

KEY WORDS: *Ovary; rhabdomyosarcoma; carcinomatosis*

CASE PRESENTATION

A 56-year-old Caucasian female patient was assessed in the outpatient surgical clinic with symptoms of abdominal distension and alteration of bowel habits with predominance of constipation. Clinical examination revealed the presence of a palpable pelvic mass extending to the periumbilical level. An urgent computed tomography (CT) scan of the abdomen and pelvis detected a 19x25cm multi-lobulated mass of probable gynaecological origin, with encasement of the greater omentum and transverse colon. Colonoscopy was unremarkable and no obvious metastatic deposits were evident on a subsequent staging chest CT, while pelvic ultrasound confirmed the gynaecological origin of the mass (Figure 1).

After informed consent, the patient was taken to the operating theatre for exploratory laparotomy, with the intraoperative findings revealing the presence of disseminated peritoneal carcinomatosis, with the described complex pelvic mass infiltrating the right and transverse colon, as well as a significant portion of the small bowel. Hence, debulking surgery was performed and the patient returned to the ward for routine postoperative care; she was discharged in a stable condition on the 6th postoperative day. The patient succumbed approximately 40 days from the operation due to pulmonary embolism, prior to discussions regarding palliative adjuvant therapy. Histopathological assessment of the extracted specimen revealed the presence of disseminated ovarian rhabdomyosarcoma of alveolar subtype, with diffuse positive staining for vimentin, 60-70% positive staining for desmin and 30-40% positive nuclear staining for myogenin (Figure 2).

Being an uncommonly encountered tumour, OARMS has an unpredictable biological behaviour and diagnosis is made after meticulous histopathological assessment [1,2]. Therefore, to the best of our knowledge, no solid consensus exists regarding its optimal treatment. In our case, upfront exploratory was performed due to the absence of non-peritoneal disease, however, due to the extensive small bowel encasement in multiple

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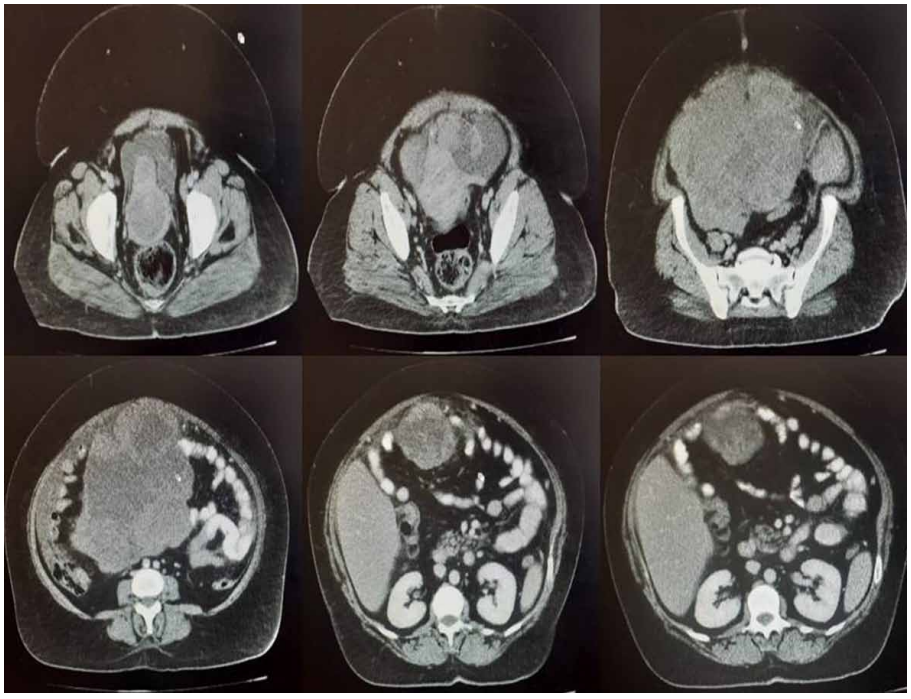

FIGURE 1. Compilation of CT images demonstrating a complex pelvic mass extending to the upper abdomen.

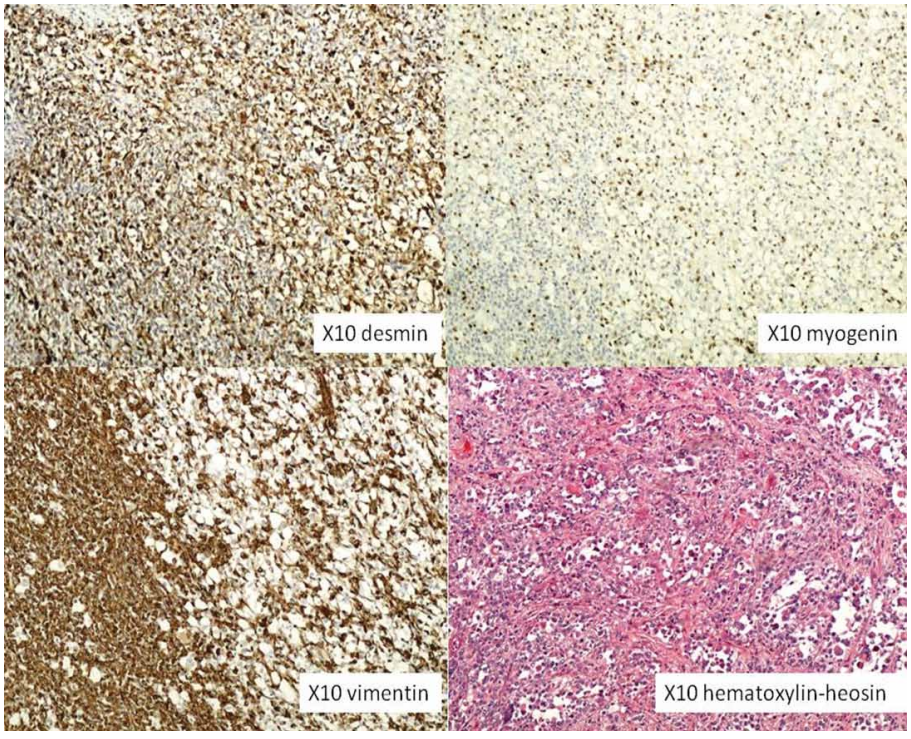

FIGURE 2. Compilation of ovarian alveolar rhabdomyosarcoma key histopathological images demonstrating -including others- positive staining for desmin, myogenin and vimentin.

levels, complete cytoreduction was not achieved and we opted for maximal tumour debulking.

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Author contributions: *NR, NB, NK and NV: contributed to the clinical data collection and prepared the case report; CS and*

GZ: contributed to the design of the case report presentation and performed the final revision of the manuscript.

Data Availability Statement: *The authors declare that the supporting data for this case presentation are presented within the manuscript*

Informed Consent: *Informed consent was obtained from the patient and is available upon request by the editorial office; no ethical committee approval was required for the publication of this case report.*

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