GRIGORE T. POPA UNIVERSITY OF
MEDICINE AND PHARMACY IASI

From old to new concepts in the systemic assessment, through an anatomic and surgical perspective

- HABILITATION THESIS –

DELIA HÎNGANU, MD, PHD

- 2019 -

CONTENTS

ABBREVIATIONS

AAMV - maximum axial amplitude of valleculae
ASMV - maximum sagittal amplitude of valleculae
CBCT - Cone-beam Computed Tomography
CRC - colorectal cancer
DAP - anteroposterior diameter of glottis
DAP/DMG - anterior posterior glotic diameter
DAT - arytenoid diameter
DC - Maximum distance between vocal cords
DECV - distance between the top of the epiglottis and the vertebral column
DEH - distance between the top of the epiglottis and the body of the hyoid bone
DEL - distance between the top of the epiglottis and the base of the tongue
DPF - maximum opening of the oropharyngeal isthmus in the palatopharyngeus muscles
DPG - maximum opening of the oropharyngeal isthmus in the palatoglossus muscles
EMVI - extramural vascular invasion
GCV - thickness of the vocal folds
GMD - Glottal maximum diameter
GP - skeletotopic projection of glottis
HIC - infraglotic floor height
HIG - Height of infra-glottal floor
HVC - height of the vocal folds
ITL - Interarytenoidian distance
LCV - lenght of the vocal folds
LIT - interarytenoid diameter
MIG - The length between the voice processes
MLH - maximum laryngeal height
mLH - minimum laryngeal height
MP - mimed phonation
MVD - microvessel density
PVP - the position of the palatine veil
SMAS – superficial musculo-aponeurotic system of the face
STI - inferior temporal septum
STS - superior temporal septum
VCL - Vocal cords length before and after mucosal removal
VIG - Volume of infraglottal floor
VPP - skeletotopic projection of the palatine veil free edge

ABSTRACT

Teaching profession is a complex one, where success is based on perseverance and desire for self-improvement, openness to new ideas and concepts, flexibility, dynamism and critical reflection. This complexity is due to the fact that a high-level university career comprises three complementary sides: teacher profession, researcher and medical profession.

The Habilitation Thesis summarizes my research activity after PhD graduation at the "Grigore T Popa" University of Medicine and Pharmacy Iasi in September 2011, confirmed by the Ministry of Education, Research and Innovation, through the Order of the Minister of Education, Research and Innovation no.6468 of 7.12.2011.

The election of the thesis title is suggestive as regarding the main features of my academic career. Thus, I am referring first of all to my belive that I have always considered surgery and medical practice as an opportunity to study the ”normal” - functional anatomy, and secondly to my permanent concern in highlighting and reasearching of the main functional aspects of the studied anatomical structures and implicitly their applicability in the vast field of clinical and radiological anatomy.

The Habilitation Thesis summarizes the scientific contributions I have achieved in these years, after the graduation of the doctoral study. If during the PhD studies I have approached and researched various techniques that allow to deepen the knowledge regarding the etiopathogenic mechanisms of the emergence and proliferation of colorectal malign tumours, in this thesis I centered the scientific contribution on the field of neovasculogenesis of the pelvic tumours.

All the research directions concern also aspects related to the higher qualified human resource in working teams and, at the same time, the impact of the dissemination of the results on the academic environment that I represent.

In this context, the first part of the habilitation thesis presents the most relevant results from the core of my researches after the PhD thesis. The results of my research transmit relevant messages to stimulate the continuation of explorations in both the main and the related areas of interest.

At the same time, I highlight the strengths and weaknesses points of their implementation so that we can "extract" a series of useful experiences and observations in order to apply for research projects and for increasing international visibility at university research level.

Ascension in higher echelons is based on the assessment of the academic level attained by current standards. The current standards required in high-level academic careers emphasize the need for continuing professional training, the modernization of teaching techniques, the continuous updating of curricular content. All these work together to increase the quality of the educational process and the results obtained.

The university teacher is an active member of the academic community and has a significant impact on it. In this sense, it must harmoniously combine qualities such as: a solid scientific knowledge, availability and pleasure to communicate with others, desire and ability to work in a team, the ability to create and coordinate work teams, the ability to identify and motivate human resources.

The habilitation thesis summarizes my professional, academic and scientific postdoctoral work and is structured in three major sections, according to the criteria recommended and approved by the National Council for Attestation of University Titles, Diplomas and Certificates (CNATDCU). The manuscript presents an overview of my concerns in the fields of interest, integrating the three sides of my career.

At the same time, the thesis contains detailed descriptions of the future research projects that I want to follow in the future.

At the beginning of the thesis I present bilingual summary. Then, in the first part - titled SECTION I - I present the results of my professional, scientific and academic activity in three subchapters, summarizing the main personal contributions in the field of functional, clinical and radiological anatomy. These demonstrate my ability to initiate and develop both personal research projects and teamwork.

These three components are: progress in the morphological study of vascular system in pelvic cancers; progress in the study of fascial structures of the face; progress in studying the singing voice.

The first chapter is the presentation of my postdoctoral activity. Here I have used the abilities and techniques that I familiarized with in the study of pelvic cancers. This novel approach of female genital cancers and digestive cancers is based on topographic criteria and those related to the etiopathogenic mechanisms of tumor neoangiogenesis phenomena.

The most important impact of the results is on the possibilities that they open up in establishing radiological protocols for early diagnosis and for staging of genital and digestive pelvic cancers.

The study of the superficial fascia of the face, with its topographical and functional features, has opened the way for deepening the anatomical substrate of the mechanisms underlying the phenomenon of aging face. The anatomical and functional study of the superficial cervicofacial muscular system (SMAS) is the starting point for research into its topographic, clinical and radiological features.

Thus, the mapping of regional SMAS characters opens the way to studying the congenital malformations of the structures that make up its structure.

The study of the opera voice, on a time criteria is the last direction of research that we have approached. The fascination and feelings that create the special vocal abilities of certain people are the main subject of this theme.

All three major research areas have paved the way for new ideas and projects that are debated in the corresponding part of the thesis.

In the second part, entitled - SECTION II - I grouped my scientific and professional achievements in four main parts: career overview; academic activity; research projects; future directions for study.

This chapter of the thesis represents the natural and logical consequence of my work so far and also with regard to future research plans and projects that I am considering. They derive from the knowledge I have accumulated so far.

In this part, I have summarized the most significant aspects of my career, which add value to my empowerment in achieving the main goal – the research at the academic level. My medical career is mirrored in academic and research through the study of colorectal cancers, the main cause of mortality in digestive oncology.

The aspiration for the continuation of the personal research projects is supported by a series of attestations obtained from the continuous medical training courses that I have followed in the field of digestive endoscopy and the medical and university management.

Most of the scientific achievements so far have materialized in book publications, articles and oral communications at congresses in the country and abroad. They hide behind them a hard work of teamwork and tenacity, contending to increase my international visibility and, directly, the prestige of the university I represent.

The topic chosen for doctoral research as well as its follow-up have opened my way to pelvic cancer research.

This section ends with the presentation of the most important achievements disseminated through articles published in ISI rated journals with impact factor.

At the end of this thesis in the chapter entitled SECTION III I attached the list of significant and relevant biographical references for assessing the current moment of knowledge in the domains of interest and those related to them.

REZUMAT

Profesia pe care am ales-o este una complexă, în care succesul se bazează pe perseverență și dorință de autodepășire, receptivitate la idei și concepte noi, flexibilitate, dinamism și reflecție critică. Această complexitate se datorează faptului că o carieră universitară la nivel înalt cuprinde trei laturi imperios complementare: profesia de dascăl, cea de cercetător și cea medicală.

Prezenta Teză de Abilitare însumează activitatea mea de cercetare de după susținerea Tezei de Doctorat în cadrul Universității de Medicină și Farmacie ”Grigore T Popa,, Iași, în luna septembrie a anului 2011, fapt confirmat de Ministerul Educației, Cercetării și Inovării, prin Ordinul Ministrului Educației, Cercetării și Inovării nr.6468 din 7.12.2011.

Alegerea titlului tezei este sugestivă pentru caracteristicile principale ale carierei mele academice, și aici mă refer în primul rând la faptul că am considerat întotdeauna chirurgia și practica medicală ca fiind o oportunitate de a studia *normalul* - anatomia funcțională, iar în al doilea rând la preocuparea mea permanentă de a obiectiva și cerceta principalele aspecte funcționale ale structurilor anatomice studiate și, implicit aplicabilitatea acestora în domeniul vast al anatomiei clinice și radiologice.

Această Teză de Abilitare sumarizează contribuțiile științifice pe care le-am realizat în acești ani, după finalizarea studiului doctoral. Dacă în timpul doctoratului am abordat și cercetat diverse tehnici care să permită aprofundarea cunoștințelor legate de mecanismele etiopatogenice de apariție și proliferare a tumorilor maligne colo-rectale, în această teză am centrat contribuția științifică pe domeniul neovasculogenezei tumorilor pelvine.

Toate direcțiile de cercetare îmbrățișate vizează și aspecte legate de componenta resursei umane superior calificate în echipe de lucru și, totodată impactul diseminării rezultatelor asupra mediului academic pe care îl reprezint.

În acest context, prima parte a tezei de abilitare prezintă cele mai relevante rezultate din nucleul cercetărilor pe care le-am realizat după susținerea tezei de doctorat. Rezultatele cercetărilor mele transmit mesaje relevante pentru stimularea continuării cercetărilor atât în domeniile de interes principale cât și în cele asociate acetora.

În același timp subliniez punctele forte și punctele slabe ale implementării acestora, astfel încât să putem „extrage” o serie de experiențe și observații utile pentru aplicarea spre proiecte de cercetare finanțate și creșterea vizibilității internaționale la nivel de cercetare universitară.

Ascensiunea în eșaloanele superioare se face pe baza evaluării nivelului academic la care s-a ajuns după standardele curente. Standardele actuale necesare în cariera academică la nivel înalt subliniază necesitatea pregătirii profesionale continue, modernizarea tehnicilor didactice, actualizarea permanentă a conținutului curicular. Toate acestea conlucrează spre creșterea calității procesului educațional și al rezultatelor obținute.

Cadrul didactic universitar este membru activ al comunității academice și are un impact semnificativ asupra acesteia. În acest sens, el trebuie să îmbine armonios calități precum: un bagaj de cunoștințe științifice solide, disponibilitatea și plăcerea de a comunica cu alții, dorința și capacitatea de lucru în echipă, capacitatea de a crea și coordona echipe de lucru, abilitatea de a identifica și motiva resursele umane.

Teza de abilitare sintetizează activitatea mea postdoctorală profesională, academică și științifică și este structurată în trei secțiuni majore, conform criteriilor recomandate și aprobate de Consiliul Naţional de Atestare a Titlurilor, Diplomelor si Certificatelor Universitare (CNATDCU). Manuscrisul prezintă o imagine de ansamblu a preocupărilor mele în domeniile de interes, integrând cele trei laturi ale carierei mele.

Totodată, teza conține și descrieri detaliate ale proiectelor de cercetare viitoare pe care îmi doresc să le urmez în următorii ani.

La începutul tezei prezint rezumatul bilingv al acesteia.

Mai apoi, în prima parte - intitulată SECTION I - prezint rezultatele activității mele profesionale, științifice și academice în trei subcapitole, rezumând principalele contribuții personale din domeniul anatomiei funcționale, clinice și radiologice. Acestea demonstrează atât capacitatea mea de inițiere și dezvoltare a proiectelor de cercetare personală, cât și pe cea de a lucra în echipă.

Aceste trei părți componente sunt:

1. Progrese în studiul morfologic al aparatului vascular în cancerele pelvine
2. Progrese în studiul structurilor fasciale ale feței
3. Progrese în studiul vocii cântate.

Primul subcapitol al acestei părți reprezintă continuarea studiului doctoral prin utilizarea abilităților și tehnicilor cu care m-am familiarizat în studiul cancerelor pelvine. Această abordare inedită și avansată a cancerelor din sfera genitală feminină și din cea digestivă are la bază criteriile topografice și cele legate de mecanismele etiopatogenice din fenomenele de neoangiogeneză tumorală.

Impactul cel mai important al rezultatelor din această direcție de cercetare este asupra posibilităților pe care le deschid în realizarea unor protocoale radiologice de diagnostic precoce și de stadializare a cancerelor pelvine genitale și urologice.

Studierea fasciei superficiale a feței, cu principalele sale caracteristici topografice și funcționale mi-a deschis calea spre aprofundarea substratului anatomic al mecanismelor care stau la baza fenomenului de îmbătrânire a feței. Studiul anatomic și funcțional al sistemului muscloaponeurotic superficial cervicofacial (SMAS) reprezintă punctul de plecare în cercetarea particularităților topografice, clinice și radiologice ale acestuia.

În mod implicit, cartografierea caracterelor regionale ale SMAS ne deschide calea spre studierea malformațiilor congenitale ale structurilor care intră în alcătuirea sa.

Studiul vocii cântate de operă reprezintă, pe criteriu temporal, ultima direcție de cercetare pe care am abordat-o. Fascinația și sentimentele pe care ni le creează abilitățile vocale deosebite pe care le au anumite persoane reprezintă subiectul acestei teme.

Toate cele trei mari domenii de cercetare menționate au deschis calea spre noi idei și proiecte care sunt dezbătute în partea corespunzătoare a tezei.

În cea de a doua parte, intitulată - SECTION II - am grupat realizările mele științifice și profesionale sub forma a patru componente principale:

1. Prezentarea generală a carierei
2. Activitatea academică
3. Proiecte de cercetare
4. Direcțiile viitoare de studiu.

Acest capitol al tezei reprezintă consecința firească și logică a activității mele de până în prezent, în ceea ce privesc viitoarele planuri și proiecte de cercetare pe care le am în vedere. Acestea derivă din cunoștințele acumulate până acum de către mine.

În această parte am rezumat cele mai semnificative aspecte ale carierei mele de până acum și care aduc un plus de valoare abilitării mele în vederea atingerii scopului vizat - cotinuarea și aprofudarea cercetării la nivel academic. Carieră mea medicală se oglindește în cea academică și de cercetare prin prisma studiului cancerelor colo-rectale, principala cauză de mortalitate din sfera oncologiei aparatului digestiv.

Aspirația spre continuarea proiectelor personale de cercetare au ca punct de sprijin o serie de atestate obținute în urma stagiilor de perfecționare medicală continuă pe care le-am urmat în domeniile endoscopiei digestive diagnostice și managementului sanitar și universitar.

Majoritatea realizărilor științifice de până acum s-au materializat în publicații de cărți, articole și comunicări orale sau tip poster la congresele de profil din țară și din străinătate. Acestea ascund în spatele lor o muncă asiduă de echipă și tenacitate, concurând la creșterea vizibilității mele internaționale și, în mod direct, a prestigiului Universității pe care o reprezint.

Tema aleasă pentru cercetarea doctorală precum și continuarea sa după finalizarea acestuia mi-au deschis calea către cercetarea cancerelor pelvine. În toată această perioadă a studiului doctoral am deprins abilitățile de cercetător necesare carierei mele viitoare și m-am familiarizat cu tehnicile de colorare uzuală și cele imunohistochimice, interpretarea rezultatelor CT și IRM. Totodată, m-am familiarizat cu tehnicile de diagnostic clinic și radiologic avansate ale acestor patologii.

Această secțiune se încheie cu prezentarea celor mai importante realizări diseminate prin articole publicate în reviste cotate ISI, cu factor de impact.

La finalul acestei teze in capitolul intitulat SECTION III am atașat lista referințelor bilbiografice semificative și relevante pentru aprecierea momentului actual al cunoașterii domeniilor de interes cât și a celor conexe acestora.

SECTION I - SCIENTIFIC ACHIEVEMENTS FROM THE POSTDOCTORAL PERIOD

1. ADVANCES IN MORPHOLOGICAL STUDY OF VASCULAR APPARATUS IN PELVIC CANCERS

Brief history of pelvic vascular apparatus

Anatomy of the cardiovascular system have been extensively studied since 3500 BC, by Greek and Egyptian scientist, although this was primarily related to religious beliefs. From the pre-Hippocratic era, the Ebers Papyrus was the the oldest anatomical manuscript in the world (Ebbell, 1937; Loukas 2016) and it demonstrated the Egyptian contribution to cardiovascular anatomy. They were the first to recognize the cardiocentric nature of the body, by identifying the heart as the center of the vascular system and relate it to the lungs and the blood (Bryan, 1930; Boisaubin, 1988). Records from 2600 B.C. show that Chinese had an early understanding of the human body(Persaud et al., 2014). They believed that the blood circulation is continuous and controlled by heart, that was also in harmony with the pulse. Unlike the Chinese, Indian scientists thought that the pulse originatedposterior the umbili-cus (Loukas, 2016; Park, 1901) and distributed blood to the whole body.

Greek scientists studied the cardiovascular system from an anatomical perspective. Alcmaeon was the first to distinguish vaguely between arteries and veins (Loukas, 2016).

The first anatomy book of the heart and the cardiovascular system, “On The Heart” is written by Hippocrates. Aristotle believed that the heart is the origin of nerves as well as both the arteries and veins (Loukas, 2016).He implied that all vessels of the body except the lungs were connected to one of the two main trunks, the “great vein” and the aorta, both originating in the heart (Harris, 1993). Praxagoras of Kos was the first to establish an absolute distinction between arteries and veins. Erasistratus, around 300 B. C. is the first to describe heart valves precisely. The discovery, description, and terminology of the tricuspid and bicuspid valves are attributed to Erasistratus. Galen disagreed with Erasistratus and held that veins originated in the liver not the heart (Harris, 1993). He differentiated between inlet and outlet valves.

In Islamic Medieval (1000 A.C) Era Avicenna wrote ”The Canon of Medicine”, where he describes blood circulation and valvular function. His most notable contribution was his correct description of the pulse, when he stated that the pulse comprises two move-ments and two pauses (Hajar, 1999). Haly Abbas provided a detailed anatomy of the heart structures based on previous discoveries. His major contribution was his description of the coronary vessels.

In Medieval European Era (1400 A.D) the most impressive contribution was made by Leonardo da Vinci. He was the first to describe the four main chambers of the heart accurately as we know them today. He also provided a detailed anatomical description of the aorta (Jose, 2001) and also important describtion of arteries structure.

In the 16th century, the major contribution in anatomy belongs to Andreas Vesalius,

with his reference book “De Humani Corporis Fabrica” (On the Structure of the Human Body). Matteo Realdo Colombo was the first to provide an anatomical description of the pulmonary circulation, and also the first to imply that blood mixes with air in the lungs rather than the heart (Tubbs et al., 2008).

In the seventeenth century the pioneers of the cardiovascular system anatomy were Wiliam Harvey, Richard Lower, and Niel Stensen. Wiliam Harvey’s major contributions were to the understanding of the blood circulation in the body (Harvey, 1962). From this period we have a detailed explanation of the pulmonary circulation, description of the muscular structure of the heart, first description of the heart as a pump, discovery of the intervenous tubercle of Lower, initial description of heart lymphatics.

In the eighteenth century major anatomical structures and pathways were discovered. The patterns of pulmonary circulation were also better understood. The major scientist with significant contributions during this era were Raymond Vieussens, Adam Christian Thebesius, Jean Baptiste de Senac, and Antonio Scarpa (Loukas, 2016).

The nineteenth and twentieth centuries were characterized by detailed anatomical studies of structures within the four chambers of the heart, and the discovery of the conducting system (Loukas, 2016).

During subsequent eras, anatomists tried to understand the true function and structure of the cardiovascular system. Many mistakes were made in the process, but these mistakes helped subsequently to elucidate the correct anatomical connections in the human body. Subsequent studies were built on these initial explanations of cardiovascular structures, until anatomists and physicians were able to relate the nature of different illnesses to the malfunctioning of different anatomical structures (Loukas, 2016).

Anatomical research in the past hundred years has taken advantage of technological developments and growing understanding of sciences such as evolutionary and molecular biology to create a thorough understanding of the body's organs and structures.

In this chapter of habilitation thesis I tried to make a systematization of my researches on the vascular apparatus, both from its involvement in the complex processes of neoangiogenesis and froman anatomical point of view.

1.1. Introduction and conceptual background

The process of tumorigenesis is closely related to the necessity to adapt the adjacent vascular apparatus to the needs of a local high blood flow. The mechanisms underlying these processes include angiogenesis and invasion of venous and lymphatic vessels around the lesion. All of this appears to take place in a certain individualized stage, depending on the topography, type and subtype of the tumor.

In this chapter I proposed to make a correlation between the complex aspects of tumor vasculogenesis in two of the most common pelvic cancers.

In the last years has increased the needing to address the management of pelvic most frequent cancers from a multidisciplinary perspective, and this is mainly due to rapid advancement of medical imaging technology. Thus, it became important for surgeons,

oncologists and pathologists to highlight the anatomical substrates identified through imaging methods in order to formulate a correct diagnosis and to determine a disease outcome.

All of these are possible by assessing morphological and topographical aspects of vascular apparatus that surrounds the tumor. Of the pelvic cancers, the highest incidencein both genders belongs to rectal cancers, and in women to cervical cancers (Edwards, 2010).

Recent data from medical literature claim that survival for more than 50% of patients with colorectal cancer might be prolonged if their disease could be prevented, or even diagnosed early in a curable stage. In the U.S. approximately 56,300 colorectal cancer patients die each year (America center in 1999).

Colorectal cancer has several features that make it ideal for screening:

- a frequently fatal disease if it is not diagnosed and treated in early stages,
- it develops from well-defined precursor lesions, colorectal adenomas, whose excision prevent cancer,
- it has a slowly progressing from a surgically curable stages to advanced and metastatic,
- thescreening tests are cost-effective and widely accessible (George, 2001).

Colorectal cancer represents more than 9% of new cases of cancer diagnosed and is the third leading cause of cancer mortality. The incidence is the same in women and men, but is higher in developed countries,being twice as many cases in Europe and North America (Ferlay, 2004). Screening provides an opportunity to detect early cancer, and also to remove precancerous polyps, preventing their development. There are evidences that screening significantly reduces mortality (U.S. Preventive, 2008), for example using fecal occult blood tests(FOBT) mortality is reduced with 15% (Hewitson, 2007). Randomized clinical trials haveused flexible sigmoidoscopy as screening method in the UK (Atkin, 2001), Norway (Gondal, 2003) and U.S. (Prorok, 2000). Colonoscopy with polypectomy also showed a decrease in the incidence of colorectal cancer in 76-90% of the cohort studies (Thiis-Evensen 1999, Brenner 2001, Winawer 1993). Although colonoscopy involves great cost, it is considered the gold standard method in early diagnosis of the disease (Winaweer, 2007; Robertson, 2005; Rule, 2006; Hewett, 2010).

Screening methods for colorectal cancer are considered to be most successful regarding prevention and early diagnosis (Edwards, 2010). Luminal screening addresses especially to polyps detections, and this is very important because the excision of rectal adenomatous polyps directly reflects in colon cancer prevention. With this purpose the most useful imagistic method is colonoscopy, with its derivations: sigmoidoscopy, virtual colonoscopy, irigoscopy. There is also used fecal occult blood test, but it has minimum result in positive diagnosis of adenomatous polyps (Burt, 2010). Detection of modified DNA in fecals denotes the presence of cancer or at least presence of one adenomatous polyp and it is a more accurate test than the other one. With all these arguments, from different reasons this test doesn't have a large practicability yet. Fecal Immunochemical Test is based on an immune reaction to human haemoglobin (Potak, 2010; Hundt, 2009; Van Rossum, 2008). The test does not require a special diet, it is more expensive than guaiac test but instead it is recommended only once a year. It has higher sensitivity for adenomas and gives fewer false positive results (Gollub et al., 2015).

All screening tests for average-risk persons should begin at the age of 50 years old. In USA first intention screening method is colonoscopy (Allison, 2010). Most recommended

tests are: annual fecal occult blood testing (FOBT), every 5 years sigmoidoscopy, every 5 years colonoscopy and every 5 years barium enema. The DNA fecal test has an uncertain interval for cancer evaluation. It is considered that barium enema should only be used if there is no possibility for colonoscopy, and some clinicians have even renounced of this test because of its numerous false negative results (US Preventive Services Task Force).

On the other hand, in the cervical cancers colposcopy is the gold standard screening method.

Abdominal and pelvis CT provides useful information regarding the presence of distant metastases and tumor extension to surrounding organs. The primary advantage of MRI is to assess the relationship between tumor and fascia that limits mezorectum, a crucial element to the feasibility of achieving total mezorectal excision. Thus the MRI can predict whether you can get a tumor-free edge by surgical resection (Klessen, 2007).

Cervical cancer is considered a "quiet" because the early stages of this disease can be completely asymptomatic. Symptoms usually occur only after precancerous lesions become cancerous and invade nearby tissues.

For a long time, cervical neoplasm remains without an obvious symptomatology, beeing "clinically mute" and should be detected by active consultation and screening, this being the time when detection ensures healing (Massad, 2018).

The most commonly used classification of cervical cancer is based on the FIGO system, making it sometimes difficult to achieve clinical differentiation between stage II of endometrial carcinoma and stage I of cervical adenocarcinoma. The clinically indicative element is the volume of the cervix (the normal uterine body and the voluminous cervix direct the diagnosis to the cervical adenoma).

It is considered that surgical staging does not play an important role in the treatment orientation. Approximately 85% of cases have limited lesion at cervix (Burgioti et al., 2016).

High-performance imaging techniques such as computer tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) or Axial Computed Tomography (CAT) can be used in certain situations, such as would be: primary tumor greater than 3 cm, infiltrative growth, concomitant pelvic mass, pregnant patient (MRI), radiologic treatment plan, visualization of very small metastatic lymph nodes (PET) (NCCN, 2018).

In patients with cervical cancer, MRI is considered to be the safest method of staging and achieving the treatment plan.

In comparative studies, the accuracy of MRI staging was 83% compared with 63% CT and 70% w clinical staging (Subak, 1995).

MRI can accurately identify stromal invasion (stage IB), presence of parametric extension (stage IIB), invasion of the vagina or pelvic wall (stages IIA and III) and invasion of the bladder or rectum (stage IV). Particularly important is invasion of parametrium, given the inoperability of the patients (Wuerthner and Avila-Wallace, 2016).

MRI offers the possibility of establishing the invasion (Kim et al., 2009). IRM allows the differential diagnosis between recurrent tumor and radial fibrosis, which can usually occur more than 12 months after treatment. This method determines the decrease in the number of procedures used for staging, including invasive, with a favorable cost / benefit ratio (Hricak et al., 2012).

The use of modern radiological techniques in the evaluation of paraneoplastic vasculogenesis processes may play a determining role in selecting the treatment protocol in any of the cases of malignant tumors investigated. Correlation of these data with those obtained intraoperatively, with immunohistochemical markers of neovasculogenesis and with remote monitoring of patients is a must in the effort to develop a protocol for the management of patients with pelvic malignant tumors.

This research direction has been materialized by publishing the following articles:

1. Hînganu MV, Hînganu D, Frâncu LL. Microanatomic aspects of arterial blood supply in rectal carcinomas – predictive models. Rom J Morphol Embryol 2013; 54(3): 561–565.
2. Hînganu D, Eva I, Stan C, Hînganu MV. Morphological aspects of the rectal neovascularization in colorectal cancer – anatomical-surgical and imaging implications. Rom J Morphol Embryol 2016; 57(1): 161-165.
3. Hinganu D, Hinganu MV, Bulimar V, Andronic D. Correlation criteria between extramural invasion of blood vessels and immunohistochemical markers in the processes of neovasculogenesis. Rev Chim (Bucharest) 2018; 69(2): 371-374.
4. Hînganu D, Stan CI, Ciupilan C, Grigorovici A, Bulimar V, Dima-Cozma C, Hînganu MV. Anatomical and immunohistochemical evaluation of colorectal cancer. Rev Chim (Bucharest) 2019; 70(1): 236-238.
5. Hinganu D, Hinganu MV, Mihalceanu E, Calin AM, Pangal A, Costachescu G, Romila A. Anatomical, imagistic and structural study of pharamagnetic substances in cervical tumors. Rev Chim (Bucharest) 2018; 69(3): 714-716.

1.2. Anatomo-surgical and imagistic aspects in rectal tumors

Colorectal cancer has become one of the most common forms of malignancy and of these, about a third of cases are localized in the rectum. Rectal cancer has a worse prognosis because it is characterized by a rate of local recurrence and a higher metastasis presence at the moment of diagnosis (Sagar, 1996). The fundamental principle of the curative treatment of these tumors remains surgery, but even that has undergone major changes over time as now is considered to be necessary a multidisciplinary team. The main purpose is individualizing the therapeutic strategy, consistent with patient and tumor characteristics (Salerno, 2006; Cervantes, 2007). Both preoperative, but especially postoperative, particular importance has quantitative and qualitative assessment of prognostic factors of patients, and in this respect intra and extramural invasion, the distance between the tumor and the mesorectum edge, the involvement of the lymph nodes, of the blood vessels, the peritoneum and of the sphincter complex, plays a primary role. Weighing all these factors, we have to analyse the best decision regarding the necessity or otherwise of the pre and postoperative neoadjuvant therapy as well as the decision on the appropriate surgical techniques (Torkzad, 2010; Smith, 2008; Ayuso, 2010).

Colorectal carcinoma occupies the second place after lung cancer as a cause of death in Western countries. Colorectal carcinoma is the most important malignant tumor of the gastrointestinal tract being considered a true "surgical disease", 92% of patients with colon cancer and 84% of those with rectal cancer undergoing surgery as the main curative treatment modality (Jemal, 2002). Rectal cancer represent about 24% of malignant tumors of the large intestine, the incidence of new cases of rectal cancer in the U.S. is estimated at 40,570 in 2004 (Jemal, 2004). Any adjuvant treatment modalities are taken into account according to the histopathological layout of the primary tumor and prognostic parameters that can be appreciated in terms of macroscopic and microscopic surgical piece.

Anatomical location of the tumor is important because it enables optimal application of an optimal staging system, as well as the differentiation of regional lymph nodes and non-regional ones, and the establishment of resection margins (Rieddle, 2003). The key issue with major implications for staging is to locate the boundary between the rectum and anal canal. Rectal tumors stage is done according to invasion in depth, while the anal canal tumors stage is depending on tumor size (TNM staging criteria) (Greene, 2002). It is now considered that the rectum is the structure that occupies 15 cm length above the pectinate line, being longer in men compared to women (Siegel et al., 2018).

Radiological anatomy studies the structures and the functions of human body through the radiological techniques, being an important part of anatomy. At the same time it represents the basis of radiology, because uses anatomical knowledge to recognize pathology, for the diagnosis and treatment of various diseases. Detections of colorectal cancers can go in two directions:

1. Tests that can detect a primary cancer.
2. Tests that can detect precancerous lesions also.

The latter one has a higher sensitivity. Flexible sigmoidoscopy (rectosigmoidoscopy) and colonoscopy are useful in inferior rectal cancer diagnosis as it can demonstrate the presence of synchronous supradjacent tumors and can detect the presence of precancerous lesions, like polyps, colonic diverticulosis (Levin, 2008; Kahi, 2009). Sigmoidoscopy is a highly effective (almost 100%) diagnosis method in cancer of distal colon, but it has no relevance in proximal cancer colon.

As I mentioned above, colonoscopy is considered the gold standard in colon cancer screening (Hewett, 2010). With all these, there are some problematic issues regarding colonoscopy, like higher costs and pacient preparation (Lieberman, 2010). Sensitivity limit of this test is until the millimeter size lesions. The test efficiency is especially on advanced adenomas (1 cm), the lower mortality by using this test being more significant in distal colon cancer than in proximal. This is because of different biological development of colon's lesions. On the distal colon are present lesions like flat adenomas, which present a diameter at least twice in height and risk for transforming in colonic neoplasm is ten time more than in pedunculated adenomas. Also at this level appears depressed lesions with high risk of transforming in cancer. Sessile polyps are newly recognized being a potential malign lesion so called hyperplastic polyp. These polyps appear frequently on the proximal colon and are usually covered by mucus. They have a serrated aspect in the superior parts of the colon in opposition with distal part, where the aspect is adenomatous. Because of this, they are called sessile serrated polyps or sessile serrated adenomas (Burt, 2010). Detection of this polyps is

a good reason for considering that proximal colonoscopy it doesn't have a higher sensitivity in colon cancer prevention (Legget, 2010).

Using of colonoscopy has a lot of advantages, like: visualizes entire length of the colon, is very sensitive and allows excision of polyps. Previous studies show that colonoscopy could prevent 65% of colorectal cancers (Brenner, 2007).

Double contrast barium enema can be used instead of colonoscopy because it has lower costs, but also has lower sensitivity. Double enema testing has been introduced as a screening method when it was found a decrease of mortality in colorectal cancer because of early detection of polyps and other cancerous lesions. Double enema allow seeing all large bowel, even in case of stenosis. It has a low risk of complication and an reasonable tolerance.

Virtual colonoscopy can replace colonoscopy at the patients that does not support colonoscopy (Johnson, 2008; Pickhardts, 2003). These can be achieved only if radiological team has competence in the implementation of virtual colonoscopy (Rockey, 2009).

CT is especially used for metastases detections. CT has a limited role in tumor staging and even by using contrasting substances it can not distinguish all the rectal's layers. In the advanced rectal cancer, the accuracy is between 79-94% and decreases to 52-74% in early cancer (Beets-Tan, 2000; Blomqvist, 1999; Beets-Tan et al., 2013).

Positron emission tomography (PET CT) can be used preoperatively to better assess the degree of metastasis. The importance of this method is in establishing the neoadjuvant protocol (Heriot, 2004).

MRI is useful in determining the tumor stage but performs better in identifying the presence or absence of the circumferential margin involvement (Klessen, 2007). After all, studies show that MRI has a higher accuracy than CT in nearby structures invasion detection (Mercury, 2006; Poon, 2005; Samdani et al., 2014)

Rectal tumors have low signal intensity on T1-weighted sequences, and this eases the differentiation of the high signal perirectal fat (Rothwell, 2010). Angiogenesis is a process underlying the homeostasis of the human body. However in some cases this physiological process can be dramatically modified (Gollub et al., 2018).

This pathological degeneration of angiogenesis towards the formation of new blood vessels which will feed some cells that begin to divide at an increasingly rate, underlies occurrence of cancers. There are incriminated here two distinct processes: transdifferentiation of cancer cells through vasculogenic mimetism and incorporation of cancer cells in the wall vessel (Carmeliet, 2011). Angiogenesis is the key element for tumor growth and for the development of distant metastasis (Folkman, 1992).

The complex processes of angiogenesis as the outgrowth of new vessels from a preexisting vascular network is fundamental to the understanding of vascularization in many physiological and pathological processes. In normal situations, angiogenesis is the process whereby new blood vessels are formed during embryogenesis, fetal development and placenta growth, for example. Under pathological conditions, angiogenesis is basic to wound healing, rheumatoid disease and thrombosis. In particular it is a key player during the initiation and progressive growth of most types of solid tumors and metastasis (Folkman, 1971; Liao, 2002), tumor cells and blood vessels forming together a highly integrated ecosystem (Taylor et al., 2014).

Blood is a complex fluid, the rheological properties of which lead to interesting feedback mechanisms during perfusion. Shear stresses generated within the capillary field by the flowing blood strongly influence vessel adaptation and network remodeling (Adam 1997). These shear stresses are instead affected by blood viscosity, the distribution of which depends upon a non-uniform distribution of thf hematocrit (the volume fraction of the red blood cells in the blood) within the host vasculature. Solid tumors are known to progress through two distinct phases of growth - the avascular phase and the vascular phase. Endothelial cells change their dormant state in fast growing state, as a result of the signals received from the tumor cells and the associated inflammatory cells and there is known a large number of components which induces angiogenesis.

The transition from the dormant avascular state to the vascular state, wherein the tumor possesses the ability to invade the surrounding tissue and the metastasis to distant parts of the body depends upon its ability to induce new blood vessels from the surrounding tissue to sprout towards and then gradually penetrating the tumor, thus providing it with an adequate blood supply and microcirculation. In order to accomplish this neovascularization, it is now a well-established fact that the tumors secrete a number of diffusible chemical substances into the surrounding tissues and extracellular matrix (McDougall, 2002).

For these reasons, the micro vascular intratumoral density was suggested as a criterion for prognosis in different types and locations of cancer, being used in evaluating the progresis of the breast cancer (Bosari, 1992; Hall, 1992;Milliaris, 1995), renal cancer (Herbst, 1998; Sabo, 2001; Lidgren, 2005), rectal cancer (Sokmen, 2001; Bakkar, 2003; Goddard, 2003), or prostate cancer (Mazzucchelli, 2000; Bono, 2002; Du, 2003).

There were described several methods for identifying tumor vessels based on the ability of endothelial cells of vascular tissue to release antigens which can serve as markers on tissue included in paraffin, CD34 considered to be the best marker for neovascularization (De la Taille, 2000).

As I mentioned above, modern management of colorectal cancer is multidisciplinary, with a major focus on preoperative assessment. This has become possible with the advancement of both medical imaging and immunohistochemical techniques. The formulation of a certain diagnosis and therapeutic protocol is a common element for aspiring multidisciplinary effort made by surgeons, oncologists and pathologists. In order to achieve preoperative staging of rectal cancer, it should be known local extension of the tumor and systemic dissemination of cancer cells. Formulation of a therapeutic protocol and prognosis of colorectal cancer depend on tumor stage, tumor type, invasion of lymph nodes, as well as whether or not the sphincter apparatus and neighborhood fascia are involved.

Neoangiogenesis occurs independently of malignant transformation, involves growth factors, extracellular matrix enzymes, endothelial cell migration and proliferation, lumen formation and anastomosis with other vessels, in relation to phenotypic and tumor genetic changes. Some authors have experimentally demonstrated that neoplastic cell populations release angiogenic molecules "in vivo", before their neoplastic transformation reaches the level of formation of a solid tumor. Tumor growth and transformation of tumor cells into an angiogenic phenotype are associated with increased secretion of angiogenic molecules, such as fibroblast growth factor, FGF and endothelial proliferation factor VEGF. In addition, it has been shown that a tumor develops a pronounced angiogenic phenotype when angiogenesis

inhibitors (anti-angiogenic factors) are suppressed during tumorigenesis, such as the case of thrombospondin, which normally helps keep the vessels in a non-angiogenic status (Park et al., 2014).

Initially, was used antibody measurement for CD31, CD34 markers and for growth factor VIII, but was recently used the quantification of CD105 endothelial cell marker expression (Ottaviano, 2015; Goldis, 2015). It seems, however, that this marker is not specific only to vascular endothelium (Agaki, 2001).

Neovascularization that crosses the tumor mass develops rapidly and presents itself as a disorganized chaotic network with tortuous, thin walls; as a result, the irrigation of different tumor segments is differentiated, with deficiencies in some segments which can lead to tissue necrosis. In this context it was found that the tumor aggressiveness is much higher in the periphery than in the tumor center, in terms of microvascular density per mm^2 (Romani, 2006).

Structural features of the vascular network in tumors are: flattened endothelium, no differentiation between arteries and veins, poor structural stability, lack of parietal contractile elements, lack of receptors, incomplete formation of basal membrane, lack of lymphatic formation (Corines et al., 2018).

Clinical studies, performed in order to verify the existence of a correlation between angiogenesis and the progression of tumor disease and to establish a possible prognostic role of the presence and quantification of the new vascular network, have produced the following results:

• metastasis in local and distal lymph nodes, as well as prognosis, were strongly correlated with the microvessel density presented in the tumor,

• because neovascularization can be visualized and quantified without using invasive methods, and since, as demonstrated, is correlated with tumor development, it can be an important tool in early detection of tumors as well as in monitoring the effectiveness of antitumor therapies (Cidon, 2016).

CD-34 is an endothelial antigen that is used to highlight microvascular density (MVD) as a direct marker of neoangiogenesis degree (Hansen, 2010).

As we noted in our study, this marker can react not only with "newly formed" vessels but also with normal vessels trapped under the tumor tissue, and overexpression of CD34 has been correlated with unfavorable progression, the transition from localized form to metastatic disease and postoperative recurrence in patients with colorectal neoplasm. It has been shown to be expressed both on the surface of endothelial cells and on the surface of neoplastic cells, their density being correlated with prognosis (Jankowski, 2008).

The emergence and growth of new capillary vessels from those that already exist is strictly addressed to the tumor, a phenomenon that is required for metastasis processes and which, if not, does not occur. Studies conducted in important groups of patients (Miettinen 2005; Nieto 2007; Da Silva 2008; Li 2008; Ma 2010) show a deterministic relationship between microvascular density and metastatic risk. The data shown indicates that the expression of the CD-34 marker is inversely proportional to the degree of histological differentiation of the tumors and to the staging of the tumor according to the degree of local extension. This immunohistochemical marker has a high sensitivity and precision, but its expression can not play the role of organ diagnosis because it can not locate the tumor.

Most of the studies (Brouwer et al., 2018) show a connection between tumoral angiogenesis and metastasies but, there are differences caused by the method used for immunological staining of vessels; vimentine has a lower specificity then VIIIth factor and this then CD34. Also there are significant differences between the degrees of differenciation, low, intermediary and high.

Endotelial cells crosses from resting to rapidly growing, due to the signals received from tumoral and associated inflamatory cells, being discovered more than 40 components which causes angiogenesis.

Because of this, the density of tumoral microvessels was proposed as a criterion for prognosis in different types and localizations of cancers, being used in appreciation of brest cancer evolution (Bosari et al, 1992; Milliaris et al., 1995), renal cancers (Herbst et al., 1998; Lidgren et al, 2005), rectal cancer (Sokmen et al., 2001), urinary bladder cancers (Bakkar et al, 2003; Goddard et al., 2003) or prostate cancer (Mazzucchelli et al., 2000; Du et al, 2003).

There were described methods to identify tumoral vessels using the capacity of endotelial cells, from vascular tissue, to eliberate antigens which could serve as markers, CD34 being considered the best for neovascularization (Taille et al., 2000).

Panendotelial CD34 marker highlights the status of colorectal carcinoma vascular endothelial, but not show the intensity of angiogenesis, because it marks both neoformate and normal vessels, preexisting vessels from neoplastic and nonneoplastic tissue (Vermeulen et al., 1996; Gee et al., 2003).

However, in many laboratories in the world is still used to assess neoangiogenesis taking into account as positive, increased densities of intensely colored elements.Therefore it is considered that reveal tumor's MVD (microvascular density) could be useful in evaluating of tumor aggressiveness (Weidneret, 1991).

P53, also known as "genome guardian" is one of the most affected tumor suppressor gene in the case of colorectal neoplasms. Mutations in this gene, appeared in the process of carcinogenesis, located on chromosome 17 occurs in more than 50% of carcinomas during the process of tumorigenesis. These mutations are linked with altered DNA replication and progression to cancer. The involve of this gene in the occurrence of recurrences and prognostics of colrectal cancer is questionable (Kahlenberg, 2003).

Some studies on groups of patients make from p53 gene and its mutation a marker of relapse and life expectancy (Kahlenberg, 2000), while other studies have not found the existence of such a role for the gene. Regarding the ordinary response to radio and chemotherapy p53 mutation showed a high degree of resistance to them. This mutation does not respond to 5-fluoro-uracil and methotrexate.

Molecular biology studies carried out on parts of resection of colorectal carcinoma correlate p53 gene mutations with polyp-carcinoma sequence. This neoplastic process begins with the activation of oncogenes (Ki-RAS), inactivation of tumor suppressor genes (p53 and Ki-RAS DCC), followed by suppressor gene mutations during progression from adenoma to carcinoma. These mutations type "missense" is performed simultaneously with the loss of wild-type allele, with a prolonged half-life, which makes these changes to be detectable immunohistochemically (Levine, 1991). Cases in which over 45% of proximal colon cancers develop "de novo", ignoring the polyp-carcinoma sequence are due to genomic instability associated with a defect in mismatch repair of DNA.

This form is called microsatellite instability (MSI iVisit). In these cases histologic tests show a poor degree of differentiation correlated with an increased mucinous content and lymphoid (crohn's-like) in the proximal colon cancers studied. The development of colorectal cancer may be in at least two different genetic pathways, depending on their location, proximal or distal to the splenic angle of the colon. For most clinicians, both left (and sigmoid) or right colon lesions are similary, only rectal cancer is considered a separate entity. This is due to the treatment differences that make from radio and chemotherapy an adjuvant treatment for rectal cancer and also for pelvic anatomical features. Thus, the presence of mezorectum creates technical surgery difficulties, plus the increased risk of local recurrence, both being serious concern (Liu et al., 2016; Jonker et al., 2017).

Originally detected as a nuclear phosphoprotein, p53, is an important regulator of normal cell growth (Pezzella,1994; Chang, 1993). It is a constituent expressed in most normal tissues, but in very small amounts, undetectable by conventional immunohistochemistry or immunoprecipitation. By contrast, can be detected large amounts of p53 in human cancers. P53 expression was demonstrated in many solid tumors, including those of breast, ovarian, endometrial and lung (Goh, 2004).

Neoplastic growth is characterized by uncontrolled proliferation well above the apoptosis rate, as well as loss of cell differentiation. For this point of view a neoplasm means a cell growth disorder evidenced by excessive cellular proliferation. Determination of this proliferation can be used in the positive diagnosis and prognosis of neoplastic lesions, and from this point of view, Ki67 is a valuable marker because is not influenced by other comorbidities such as melitus diabetes, cardiovascular disorders, congenital malformations. Overexpression of this marker may suggest a disturbance of cell division, resulting in the appearance of an intensely-growing tumor. The marker is also used to determine the degree of aggressiveness and the metastatic tumoral potential (Mirnezami et al., 2013; van der Geest et al., 2015).

The mechanisms by which Ki-67 is involved in cell cycle control are not fully elucidated, but there is a hypothesis that increased values of Ki-67 would act by inhibiting the cell cycle by inducing cellular self-stabilization (Mcleod,1999; Jankowski, 2008; Alberda et al., 2018).

Immunohistochemical markers CD-34 and Ki-67 appeared relatively recent in the studies. Both markers are sensitive to the assessment of tumor aggressiveness. In normal tissues are very low expressed, and abundant in tumoral tissues. Neoangiogenesis refers to the growth of new capillary vessels from those that already exist. These vessels are strictly orientated to the tumor. This phenomenon is necessary for the tumoral metastasis and, if it does not occur, it is not realized; there are studies on important batches of patients (Miettinen 2005; Ma, 2010; Nietto, 2007; Da Silva, 2008; Li, 2008) showing a relation of determinism between microvascular density and metastatic risk. The expression of these markers is correlated, in the same studies with the patients prognosis, being directly proportional to it. The same was observed in breast cancer, stomach cancer and brain cancer.

The data show that expressions of CD-34, Ki-67 markers are inversely proportional to the degree of tumor histologic differentiation and with tumor staging depending by degree of local extension. At the same time, it is widely accepted that angiogenesis processes have a determining role in the metastasis process and patient prognosis by fascial tunnels organized

arround bloodvessels. These two immunohistochemical markers are of high sensitivity and precision, but, however, their expression can not play the role of organ diagnosis because they can not locate the tumor (Riihimaki et al., 2016; Homan et al., 2015; Jonker et al., 2017).

A 6-month, serial evaluation can play the role of a marker of cancer progression or regression and signifies a new tool in assessing the prognosis of these tumors.

Within this context the objectives of our studies were to emphasize the anatomical criteria in more realistic preoperative evaluation of a patient suspected of having rectal cancer. We evaluated the degree of the extramural invasion of the blood vessels (EMVI) and thus, the impact of this determination in diagnosis, treatment and prognosis of colorectal neoplasia setting and we have also studied the immunohistochemical expression of the Ki-67, CD34 markers in colorectal adenocarcinomas in order to achieve a better prognosis.

1.2.1. Materials and methods

The study was conducted on a group of 34 patients colonoscopic diagnosed with rectal cancer, who underwent preoperative contrast MRI to establish tumor localization and resection possibilities.

We analysed the vasculature of colorectal carcinomas quantifying high density neovascular areas, using ***three different methods***, each one applied by default topographies of moderately differentiated rectal carcinoma (upper, middle and lower).

1.2.1.1. ***MRI study of extramural vascular invasion***

From the studied group we considered ten patients for the radiological study of extramural vascular invasion. The MRI images acquired on these patients were compared with normal anatomical imaging procured on a control group of 15 patients. Of the 10 patients in the study group 7 were male, aged 46-57ani and three females aged 55-62 years. Of these, 5 were diagnosed with cancer of the higher portion of the rectum in T2, T3, T4 stages, three were diagnosed with medium rectal tumors in T3 and two with lower rectal cancer, T1 and T2.

The MRI sequences obtained are consistent with others found literature (Mercury, 2006; Mercury, 2007):

- T2 fast (turbo) spin- echo, made from one pelvic wall to the other (transverse);
- Axial, with a wide field throughout the pelvis;
- T2 fine sections in the axial plane - made along its axis, that include rectal tissue with 3 mm thickness;
- Coronal sections with high spatial resolution that alow the visualization of low rectal tumors, the *levator ani* muscle and of the anal mechanism sphincter;
- High resolution Sections that go up to 5mm above the edge of the tumor in order to be able to view a possible a lymphatic tumor invasion in the mesorectum.

The MRI examination was performed using the area antennas after following the protocol: images weighted in T2 FSE and T1 SE and ESF in three orthogonal planes - axial , coronal and sagittal – with the following parameters: TR 400 msec , TE 130 msec , 400X500 matrix 28 mm FOV sections of 4mm exam T1 -weighted images natively and ESF (fast spin echo) after administration of intravenous paramagnetic contrast MIP and 3D reconstructions followed. For the study, we used a Philips Achieva 1,5T MRI machine. A paramagnetic dye

was injected: Magnegita 500 micromol/ml solution for injection (dimeglumin egadopentetate). The MRI dosage used for adults was of 0.5 ml/kg. The injection of the contrast (30 to 40 ml of Gadolinium) was carried out with a MRI specific injector (MEDRAD Spectris®) at a rate of 0.8 ml/s. For the acquisition of bolus MRI angiography we used sequences followed by successive stages: the EF- GRE 3D sequence used with the following parameters : 42 cm , matrix 256 × 192 (reconstructed by interpolation matrix 512 × 512), TE 1.5 ms, TR 6 ms, TI: 25ms angle: 25°, section thickness: 4 mm (rebuilt in pieces that overlap 4 and 2 mm).The total MRI acquisition time for the angiography was of 95 seconds (5 seconds between each step to move the table).

We used the EMVI score correlated with MRI imaging in order to establish the staging of colorectal carcinomas.

The criteria taken into account by the EMVI score were correlated with the images obtained in patients from the study group

1.2.1.2. ***Evaluation of neoangiogenesis through triangulation and Monte-Carlo method***

We have also performed quantitative evaluation of the neoangiogenesis process on the rectal neoplasm using the angiography method on the resection specimen. We applied the methods of calculating the length of the neo-angiogenesis vessels, the calculation of area of development of neo-angiogenesis through triangulation and Monte-Carlo method, and the techniques applied on angiography images of resection specimen.

1.2.1.3. ***Immunohistochemical evaluation of angiogenesis***

From the studied group 24 patients diagnosed with colorectal cancer were imunohostochimistry evaluated. We correlated the findings with imaging, intraoperative and pathologic examination, in order to establish a prognostic evaluation criteria. We made the histopathological and immunohistochemical evaluation of patients in the study group. The evaluation was performed on the sections stained with hematoxylin-eosin, and for mucinous forms, on sections stained with Alcian blue. We selected the cases for the immunohistochemical examination, the techniques being applied to low differentiated colorectal carcinomas. Ki-67 is expressed throughout the cell cycle except for the G0 phase, which is why Ki-67's intratumor concentration values correlate with the tumor proliferation rate and thus the prognosis of the patients.

The nuclear accumulation of the Ki-67 protein was assessed semi-quantitatively: negative (-) which means lack of coloration or presence in less than half of the cells and positive (+) which means the presence of nuclear staining in over 50% of tumor cells. In turn, the positive response was assessed low positive, moderately positive and strongly positive (+, ++ and +++).

The vascular endothelium was shown immunohistochemically with monoclonal antibodies CD34, class II, code M7165, Mo Hu (Dako). After qualitative examination and image acquisition 200x magnification, it was quantified microvascular density in the area of active angiogenesis, assessing the sensitivity and specificity of the endothelial marker CD34. We used filters for color segmentation and obtained intense brown color to the vessels in contrast with the background image. Necrotic areas were not included in the analysis.

Quantitative measurements were made with a digital video interactive software products (5.0.) which uses parallel Weibel grid, with the distance between two points d =

19.39 μm. it was quantified tumor stroma vessels on a rectangular surface (115.72 μ/182.45μ) with 0.0021112 mm2 area, on 10 consecutive fields, on 5 cases of each lesional type. Microvessels were counted in areas with the highest density ("hot spot"), after identifying those areas with low magnification.

Stereological quantification report calculated automatically: density of microvassels/mm^2 of tumor stroma and density of tumor microvassels/mm^2 of tumor, in the same time with the statistical verification of results.

1.2.2. Results

1.2.2.1. Results of the neoangiogenesis evaluation through triangulation and Monte-Carlo method

In the first processing stage we made the imaging processing of captured images from ”in vitro” angiography, and then we calculated the length of the most important neoformation vessels. It was found that in the superior rectal cancer the maximum length is 1019.2157 mm (Figure 1.1.), in the middle rectal cancer it is 924 mm (Figure 1.2.), in inferior rectal cancer it is 670.3964 mm (Figure 1.3.). The progressive decrease of the length of neoformation vessel stands out in tumors from the superior part of rectum, toward the middle and then the lower part.

The measurements revealed that the higher length was quantified on upper rectal cancer, followed by the middle and lower.

The following stage was the application of the triangulation method only on the areas with high density of neoformation vessel. In this case we obtained a value of the area of maximum density of neoformation vessels, as it follows: in the superior rectal cancer it is 475987.6199 mm^2 (Figure 1.4.), in middle rectal cancer it is 380084.6006 mm^2 (Figure 1.5.) and in inferior rectal cancer it is 99703.2271 mm^2 (Figure 1.6.).

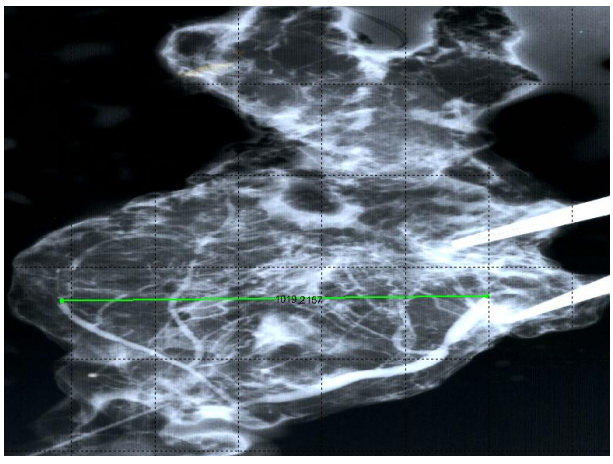

Figure 1.1. The length assessment of the most important neoformation vessel in the superior rectal carcinoma.

The triangulation method showed a progressive reduction in the value of the area with maximum density of neoformation vessels in the same direction, from the upper rectal cancer, to the middle rectal cancer and then to the lower rectal cancer.

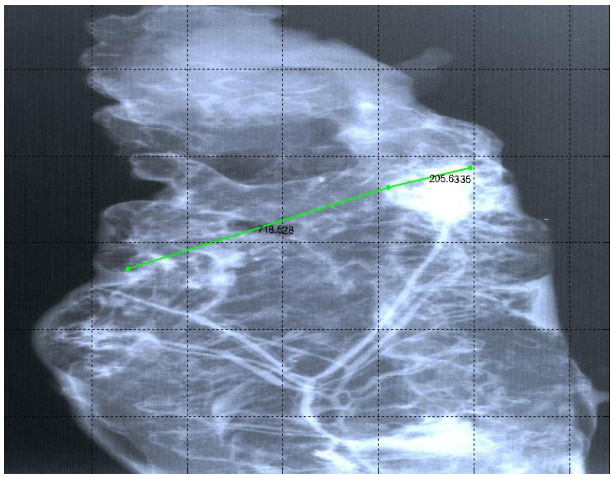

Figure 1.2. The length assessment of the most important neoformation vessel in the middle rectal carcinoma.

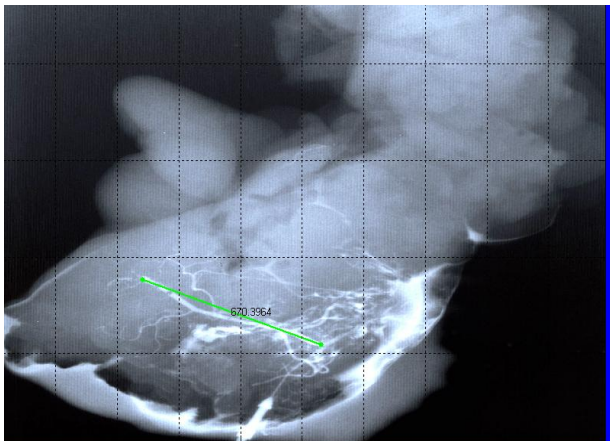

Figure 1.3. The length assessment of the most important neoformation vessel in the inferior rectal carcinoma.

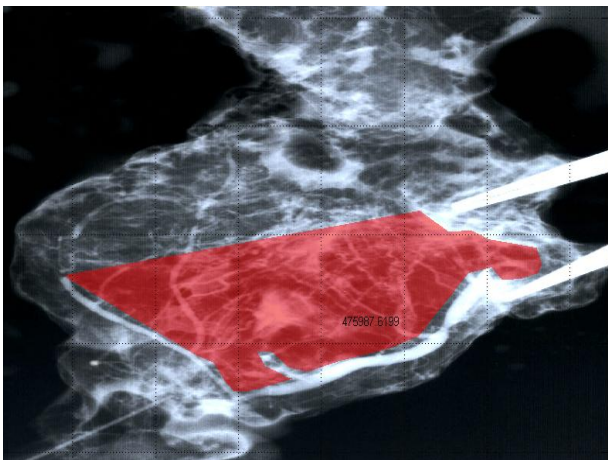

Figure 1.4. Triangulation applied on the superior rectal carcinoma.

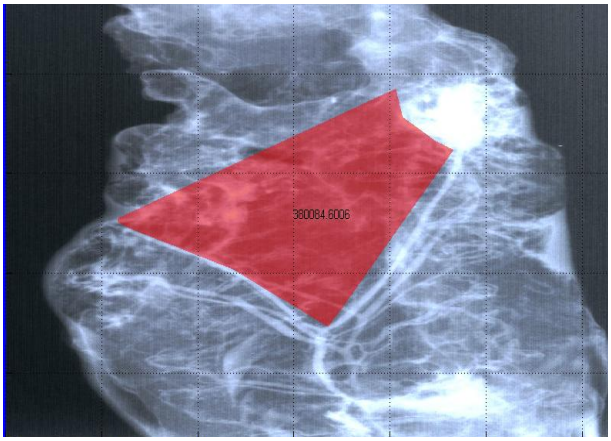

Fig. 1.5. Triangulation applied on the middle rectal carcinoma.

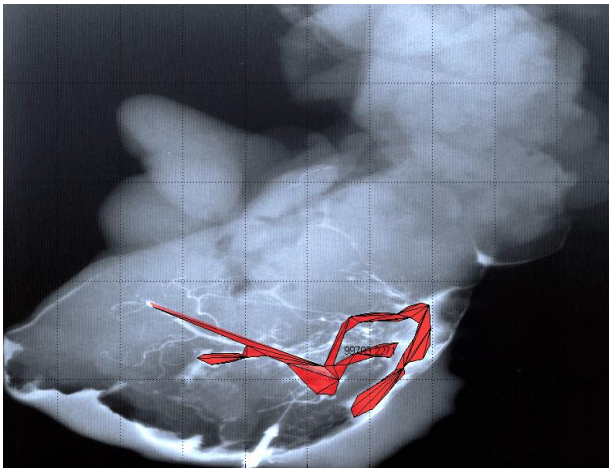

Fig. 1.6. Triangulation applied on the inferior rectal carcinoma.

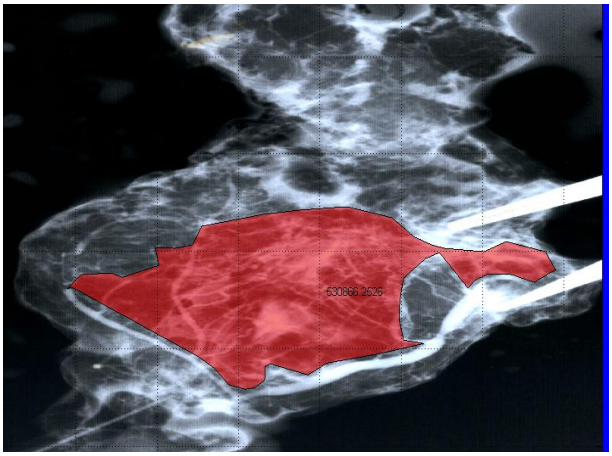

Figure 1.7. Monte-Carlo method applied on the superior rectal carcinoma.

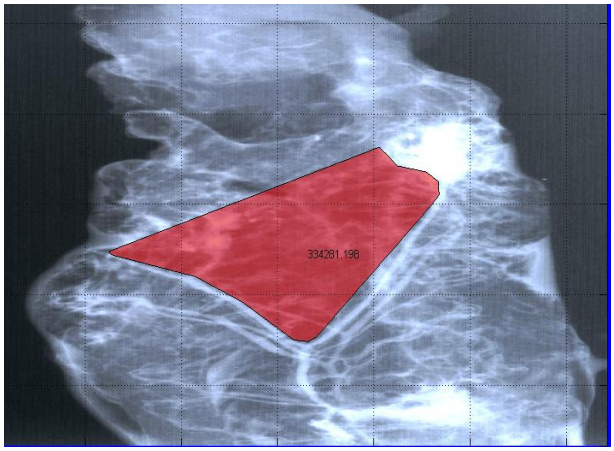

Fig. 1.8. Monte-Carlo method applied on the middle rectal carcinoma.

In the last stage of the study, on the same areas of maximum neovascularization we applied Monte-Carlo method to calculate it. The results are very close to those obtained by triangulation method: on the superior rectal neoplasm we obtained a value of 530866.2526 mm^2 (Figure 1.7.), for the middle rectal neoplasm the value is 334281.198 mm^2 (Figure 1.8.) and for inferior rectal neoplasm is 116990.2554 mm^2 (Figure 1.9.).

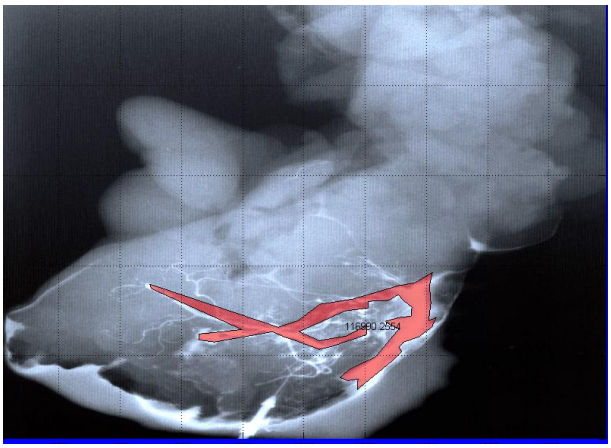

Fig. 1.9. Monte-Carlo method applied on the inferior rectal carcinoma.

Monte-Carlo method showed a progressive reduction in the value of the area of maximum neovascularization on the same sense, from the superior rectal cancer, to the middle rectal cancer and then to the lower rectal cancer, the changes being significant and similar to those obtained by the triangulation method.

In middle rectal carcinomas we applied the Monte Carlo method to make a determination on the area uncompromised by neoangiogenesis (Figure 1.10.).

In this situation normal area of rectal wall is approximately 4.5-5 times smaller than the area of neovascularization. The other two types of rectal carcinomas shows different aspects: in superior rectal carcinomas we did not found on the images processed a zone without neoformation vessels, and in inferior rectal carcinomas is a vice-versa situation.

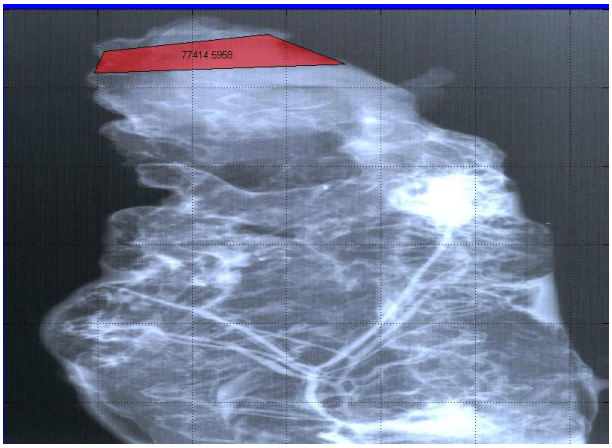

Figure 1.10. Normal rectal area.

1.2.2.2. Results of the MRI study for EMVI evaluation

In each of the evaluated patients we found both, arterial and venous blood vessels in 0 stage - EMVI correlated with T2 or T3 stage tumor. In T3 B stages, we could objectify suggestive images for stage IV EMVI near and distant from the tumor. Thus, T2 in stages (Figure 1.11.), proximal and homolateral of the tumor we have objectified the vascular paraneoplastic invasion appreciated by the caliber of the blood vessels , the degree of tortuosity , the appearance of their walls (nodule) and heterolateral the vessels appearing to be normal , especially in the early T1 and T2stages. In T1 stages, on sections made at 3mm , the paraneoplastic changes of their walls and their content lessen and even disappear at 6mm maximum, both homo and controlateral of the tumor. In T2 and especially in T3 stages, the vascular paraneoplastic changes seem to interest long vascular portions with an important caliber, going even to the origin vessels (the trunk of the rectal middle artery, the internal iliac artery of the tumor and, especially the veins of this path - Figure 1.12.).

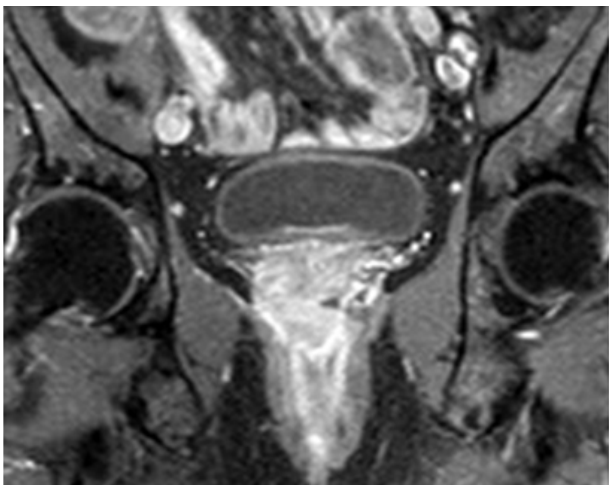

Figure 1.11 Neoformation vessels in the inferior right rectal artery, in straight angle, with enlarged perivascular lymphadenopathy - stage 2 EMVI

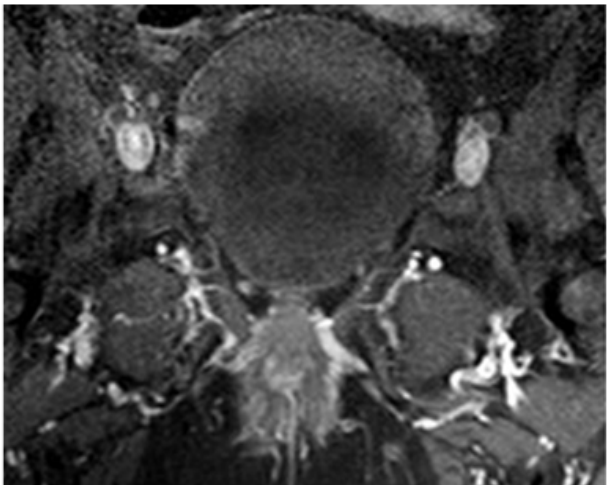

Figure 1.12. Middle rectal vein in middle rectal cancer, with the equivalent signal outlet to the tumor, specific for EMVI stage 4

The remote damage of the blood vessels (internal iliac vein) is correlated in most cases with secondary determinations of the liver (Figure 1.13.). We believe that the distance paraneoplastic impairment of the vessels (the trunk of the middle rectal artery) signifies a high probability of microscopic liver determinations.

Figure 1.13. Neovascular degenerative process, on the branches of inferior left rectal veins, with the appearance of a central core and two satellite sanguine cores, typical for arterial paraneoplastic processes - stage 2-3 EMVI

The aspect of tumor margins shows tumoral invasion in small veins, which goes out of the intestinal lumen and can produce nodules in the venous wall, distinct from desmoplasia (Figure 1.14.). The presence of tumoral signal in the vascular structures is a landmark for tumoral presence (Figure 1.15.). Affected vessels increase their volume and their inside signal is a medium gray.

Extension of tumor inside the vessels cause a smooth, nodular or an irregular appearance of vessels (Figure 1.16.).

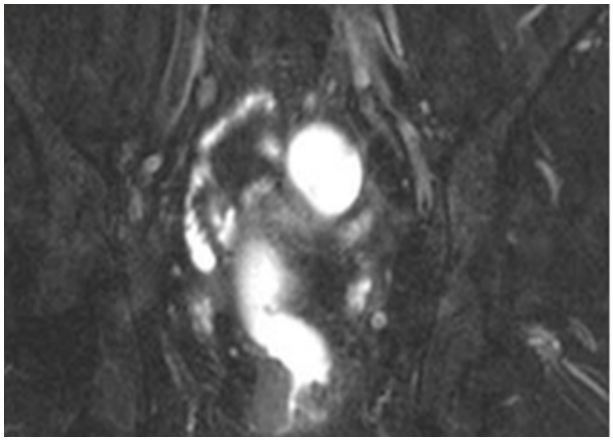

Figure 1.14 Pseudo inferior rectal artery stenosis in the left, with similar changes on the controlateral inferior rectal artery, both intramural nodule type, specific to stage 3 EMVI

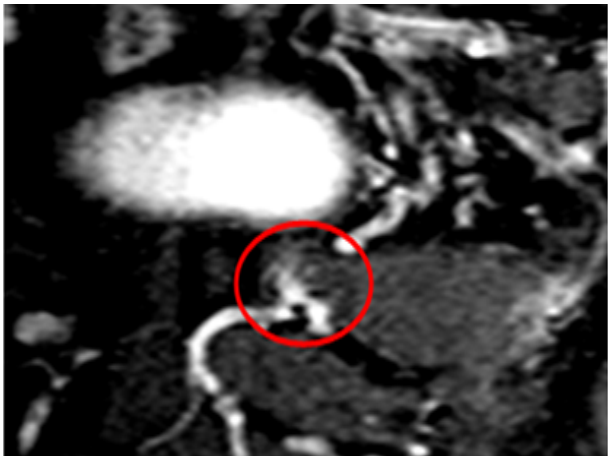

Figure 1.15. Left middle rectal vein in sagittal section, in a middle rectal cancer with intraparietal invasion - stage 4 EMVI

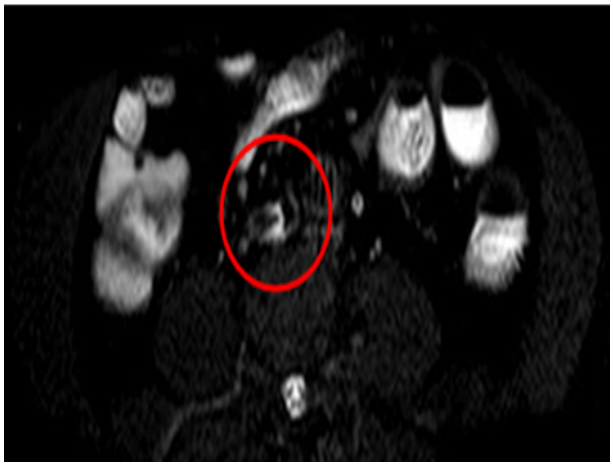

Figure 1.16. Neoformation vessels on the right inferior rectal artery, in MRI with multicenter condensing processes, characteristic for stage 3 EMVI

In none of the 15 cases of MRI investigated patients who refuted rectal neoplasia could we amend vascular corresponding EMVI score (Figure 1.17.). Moreover, in these patients we have not discovered any benign formations which can be considered preneoplastic condition, such as- polyps , diverticulum, or autoimmune diseases.

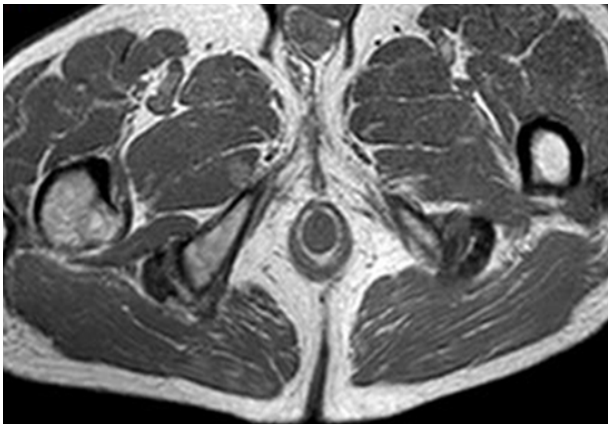

Figure 1.17. Normal aspect - stage 0 ENVI

Of primary importance in determining the localization of the tumor is the fact that the extramural invasion of the blood vessels in the lower rectal tumors is cranio-caudal and those of the upper and middle rectal tumors are mostly cross bared. This explains the rapid bloodstream metastasis of the lower rectum tumor. In this situation, the medium or large distance vascular invasion is precocious.

Upper and middle rectal neoplasms produce a precocious extramural invasion in proximity. The most used directions in extramural vascular invasion in various locations suggest the fact that they follow the vascular anastomoses between the 3 rectum territories.

Given these considerations it may be proposed an EMVI staging system based on MRI imaging.

1.2.2.3. Results of the imunohistochemical study

From the cases that were imunohistochemicaly analized for Ki67, most of them were moderate and low differentiated colorectal adenocarcinomas (G2 and G3). Approximately 60% were in advanced stages (T3), with perirectal adipose tissue invasion.

In G2 adenocarcinomas (Figure 1.18.), tumor cells delineate glandular, irregular cavities sometimes with papillary excrescences of different shapes and sizes. The nuclei are large, elongated, hypercromatic, placed on 3-4 layers that occupy the entire thickness of the epithelium. There are areas of ulceration and infection with the presence of stromal inflammatory infiltrates.

In G3 adenocarcinomas, tumor cells are usually small with hyperchromic nuclei. The stromal volume is increased, there are frequent ulceration areas, the stromal inflammatory infiltrate being abundant (Figure 1.19.).

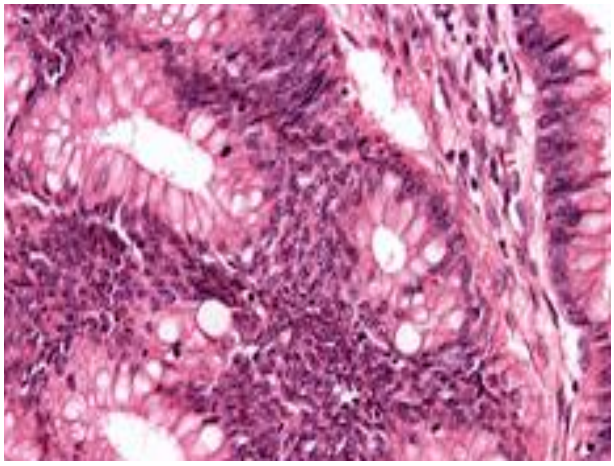

Figure 1.18. Moderately differentiated colorectal carcinoma.
H&E staining, ob. x40.

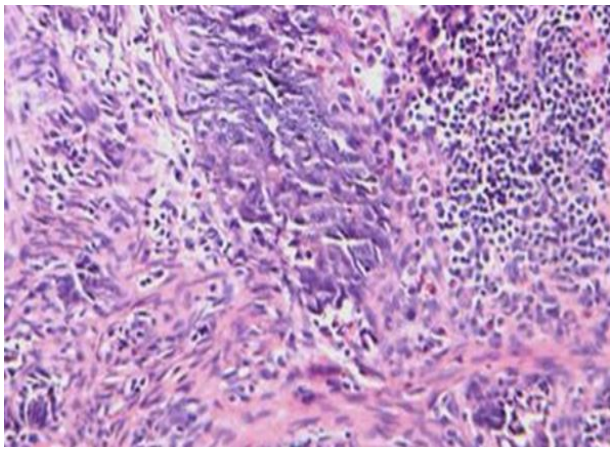

Figure 1.19. Low differentiated colorectal carcinoma.
H & E staining, ob. x20.

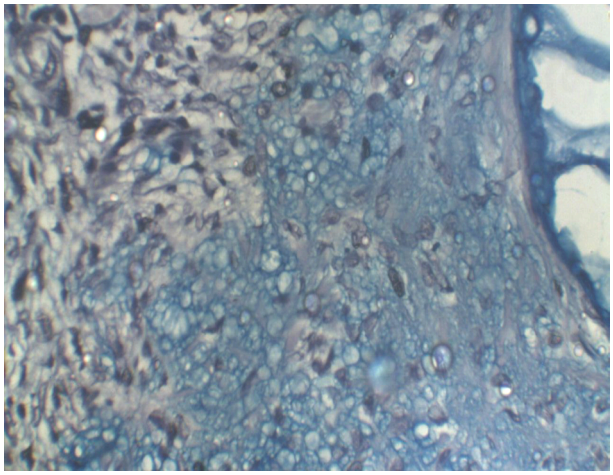

Fig. 1.20. Low differentiated colorectal carcinoma.
Blue Alcian staining, ob. x40.

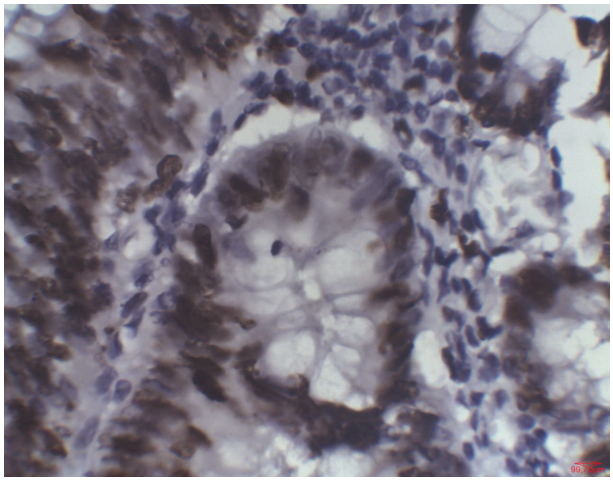

Figure 1.21. Strong nuclear reaction of Ki-67 on sections of colorectal cancer (+++). Ob. X60.

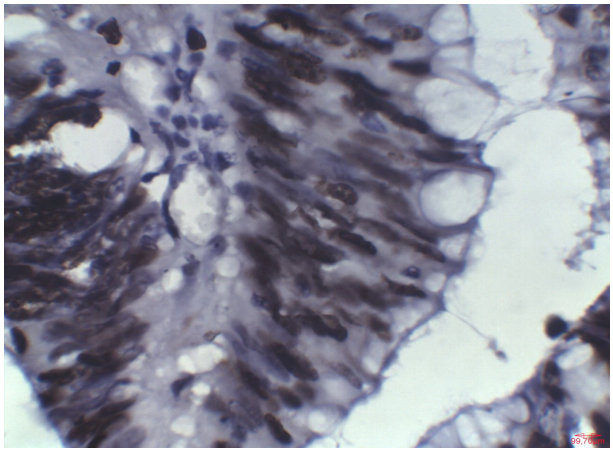

Figure 1.22. Nuclear Reaction of Ki-67 in the Cells of a colorectal cancer. Ob. X60**.**

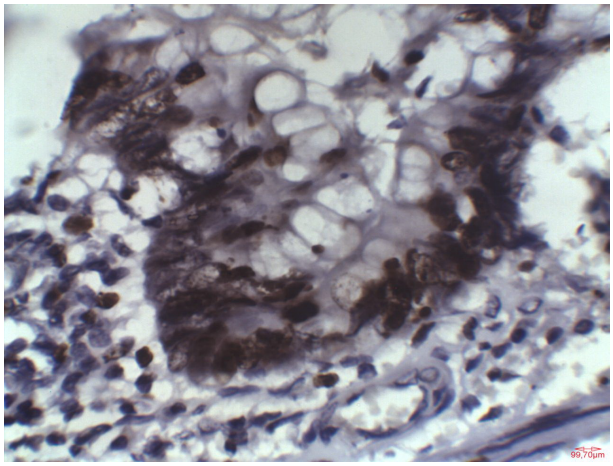

Figure 1.23. Intense nuclear (+++) reaction of Ki-67 on sections of colorectal cancer.

Of all the cases of studied colorectal tumors, we considered mucinous carcinomas the cases where the mucinous component represented more than 30% (Figure 1.20.).

Immunohistochemical exam in sections stained for Ki-67 has shown that the topography of reaction is strictly nuclear (Figures 1.21., 1.22., 1.23.), but we could not accurately identify the nuclear localization depending on the division stage.

In the tumor there are Ki-67 poor stained areas, probably due to the completion of phase (interphase) and degradation of nuclear staining.

The lack of expression of studied markers in neighboring non-tumor tissues, including Ki-67, suggests that cancerous tissue proliferates in a pathway that is not correlated with adjacent tissues.

In cases with a strong positive response to Ki-67 (+++), prognosis is worse, with invasion of the perirectal tissues, regional lymph nodes and metastases. In these situations there is a direct correlation between the expression and accumulation of the Ki-67 protein and the local, regional or distant extension.

We have not noticed any correlation between the intensity of the reaction and the age and gender of the patient or the exact location of the primitive tumor.

CD34 immunohistochemical examination of stained sections showed that vessels are present in the tumor stromal tissue without notice any reaction with tumor cells. It was observed the existence of various densities of microvassels in different tumor types, depending on the degree of differentiation.

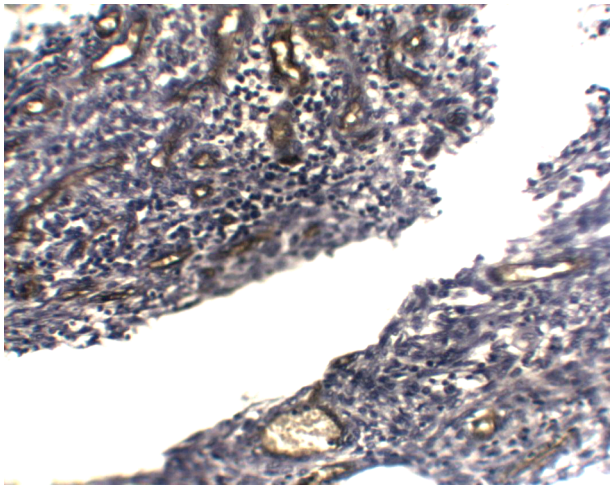

Figure 1.24. Overall appearance of the tumor stroma in colorectal carcinoma poorly differentiated in the presence of CD34 positive vessels. x200.

Each cell or endothelial cell islands in brown color, which is clearly separated from adjacent microvassels, tumoral cells and other connective tissue elements, it was considered a single quantifiable element. Lumen existence is not required to identify a structure as vascular.

In normal tissue, vessels are homogeneously distributed in the periglandular stroma, more evident around large and medium-sized glands, which make up a network.

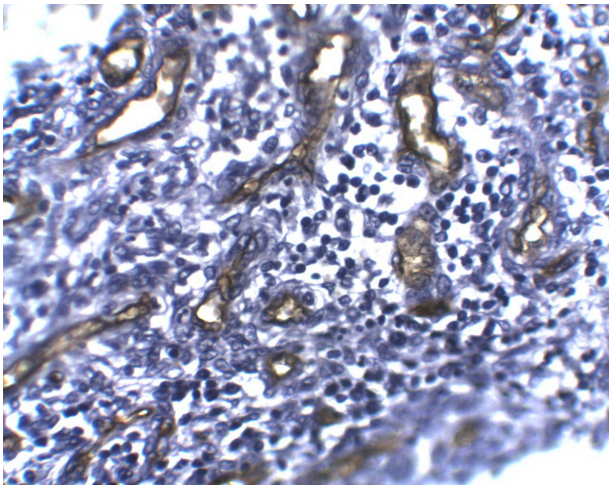

Fig. 1.25. The presence of mature vessels of various sizes and torsions, with CD34 positive endothelium. x400.

On section, most vessels have long axis parallel to the basal membrane of glandular epithelium. In carcinomas, neoformation vessels are located both intratumoral and peritumoral, variable in size and shape section (Figures 1.24., 1.25., 1.26.), their appearance ranging according to the degree of differentiation. Vessels are located mainly in stroma surrounding the tumor islands (Figures 1.27., 1.28.).

Moderately differentiated carcinomas, but especially the undifferentiated or poorly differentiated has many irregular, tortuous, and dilated microvessels (Figure 1.29.).

Neoformation of small vessels are between islands of tumor cells and can be studied away from the tumor or peritumoral, intrastromal (Figures 1.27., 1.28.).

In tumors, thin vessels can be associated with or without lymphocytic inflammatory infiltrate and take even the appearance of clusters based on atypical glands. The density of these vessels increases inversely with the degree of tumor cell differentiation.

Peritumoral small vessels are distributed at the periphery of tumor islands, and can take even look as a dividing line high degrees undifferentiated. Intrastromal number of these vessels also varies. Their density can be considerable even in cases with peritumoral fibrosis.

Over time were performed and imagined various screening methods for early detection of colorectal cancer, methods that were limited in terms of sensitivity, cost, risks and inconveniences. It started from test screening for fecal occult blood and it reached to the measurement of various biomarkers, including the CEA. The quantify of Carcinoembryonic antigen has a low sensitivity and specificity in the diagnosis of colorectal carcinoma. Hence the need for a secondary test to give better results.

CD-34 is an endothelial antigen used to highlight the microvascular density (MVD) as a marker of neoangiogenesis degree. As I said at the beginning, this marker can react not only with vessels "newly formed", but also with normal vessels trapped in tumor tissue.

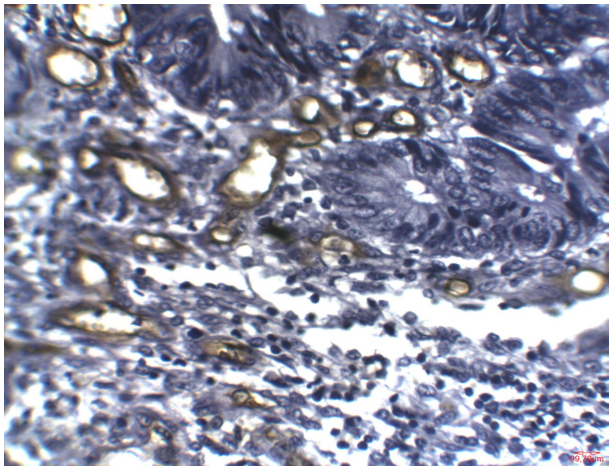

Figure 1.26. Mature large vessels of various sizes, labeled with CD34, located in the tumor stroma.x400.

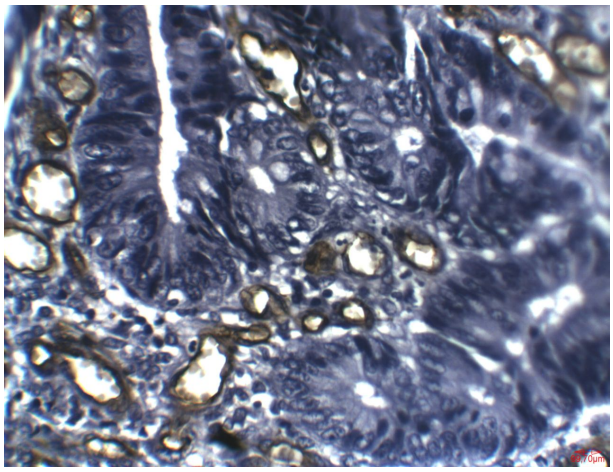

Figure 1.27. Vessels of various sizes, mature, situated in stroma of a colorectal adenocarcinoma with intense CD34 positive endothelium. x400.

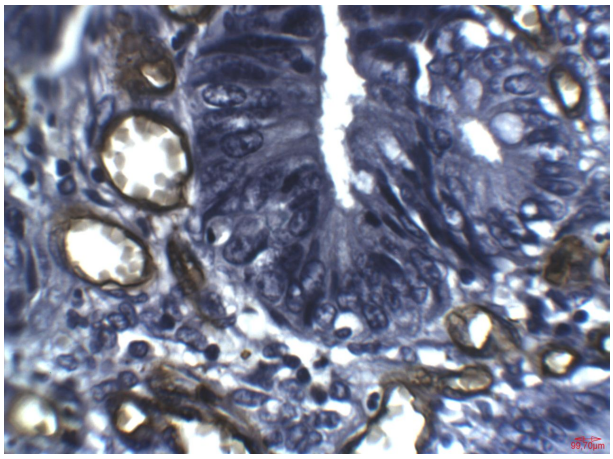

Figure 1.28. Superexpresion of CD34 in vascular tumoral endotelium. Ob. 600.

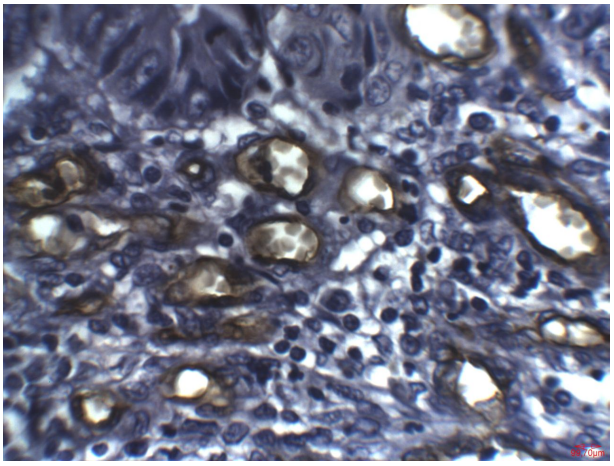

Figure 1.29. Neoformation vessels endothelium strongly positive for CD34. X600

Depending on the degree of adenocarcinoma differentiation, ranging from the well-differentiated to the poorly differentiated form, the density of the microvessels varied as follows: the microvascular density/mm^2 tumor stroma progressively increased from 480.30/mm^2 to 875.92/mm^2 and 1495, respectively, 37/mm^2, and the microvessel density/mm^2 tumor exhibited the same change of magnitude, from 670.54/mm^2 to 1053.84/mm^2 and 2250.69/mm^2 tumor respectively (Table 1). This microvessel density (MVD) criterion was commonly used as a prognostic marker in immunohistochemical determinations performed with other substances, such as VEGF A, CD105, Ki67.

Table 1 Corelation between thedegree of adenocarcinoma differentiation and the microvascular density

Vascular density	**TUMOR TYPE**		
	Well-differentiated adenocarcinoma	Moderately differentiated adenocarcinoma	Undifferentiated adenocarcinoma
Density of microvessels / mm^2 of tumor stroma (DM / ST)	480,30	875,92	1495,37
The density of microvessels / mm^2 of tumor (DM / T)	670,54	1053,84	2250,69

The correlation between measuring MVD in immunohistochemical determinations with CD34 antibody in colorectal cancers and evaluating the same patients according to EMVI staging criteria is the main novelty of this study. Both methods provide information about neoangiogenesis processes in colorectal paraneoplastic syndrome.

The association of this information increases the diagnostic and prognostic value of the two determinations - the weaknesses of the immunohistochemical evaluation with CD34 are counterbalanced by the information provided by EMVI staging and vice versa. We

consider that the simultaneous use of immunohistochemical markers linked to vascular endothelial proliferation with quantitative and, above all, qualitative assessment of tumor and peritumoral neovasculogenesis can be used as a protocol for the individualization of pluridisciplinary colorectal cancer treatment.

1.2.3. Discussions

The current direction in the management of rectal cancer is to improve existing imaging techniques and to impose new techniques in order to obtain images with high accuracy. For this purpose it is considered the decrease of image acquisition time, and also of protocol for patients training and the possibility of obtaining functional images. Knowing the advantage and limitations of different methods it can be raised up the standard of patient care and it can be improved the work in multidisciplinary teams (Taylor et al., 2014; Brouwer et al., 2018).

Paraclinical investigation of a patient who present with clinical symptoms suggestive for colorectal tumor pathology follows a specific protocol. It starts with the basic techniques (X-ray, colonoscopy) capable of detecting the presence of tumor formation. It continues with advanced techniques (CT, MRI, PET-CT, colon video capsule) which have the advantage of being able to provide information about local tumor extension, type and distance metastases (staging). Also they provide important data for determining a properly operating protocol suitable to each case. At the same time, these data are used to establish a prognostic for the patient (Gollub et al., 2018; Fujita et al., 2017).

Conventional x-ray exploration, providing anatomical and morphological information about tumor formations, continues to be the main method of exploration in most radiology departments because it is easy to make, it is cheap, and it is achieved using a low dose of radiation. At the same time it provides a significant amount of information. In terms of imaging (MRI), the examination of the pelvis and, rectum in particular, can normally display a number of ligamental structures (presacral fascia, sacrogenital ligament and Denonvilliers fascia for male patients), mesorectum, hypogastric vessels and their branches, as well as the superior and middle rectal vessels (Lee et al., 2018).

Relations between rectum and perirectal fat, urinary bladder, prostate in males, uterus and vagina in females are also visible in native exploration or using contrast agent. Other elements, such as Waldeyer's fascia, lymph nodes and lower rectum vessels are especially visible in pathological situations.

Anatomical properties, like different structure of the rectal wall, peritoneal attachments and the presence of rectosacral space, create conditions for a proper MRI exploring at this level.

In studied cases, all the imaging discoveries have been confirmed directly in surgery exploration, as well as indirectly through various technical difficulties because of development of perirectal paraneoplastic processes. In lower rectal neoplasms, there can be found deviations from a normal rectal artery (superior, middle and/or lower) that seem to "dress the tumor".

Neovascularization is characterized by multiple arterial branches, in right angles, with spider web appearance in some cases, more dense (convergence) to the tumor core.

Most of these tumors have stenotic character, subocclusive or even occlusive with late clinical signs.

In neoplasms of lower rectal, the proximity and "early catching" of vessels, first in perilesional edema, and then in the extensive tumor, give the capital clinical sign of rectorrhagy (through ulcers).

Quantification of the maximum neoangiogenesis area realized throughout the former 3 mentioned methods sustains high potential for metastasis via blood flow of the superior and the inferior rectal tumors and explains their larger size compared to the inferior rectal ones.

Comparison of micro quantifying on histological sections, previously performed, with micro vascular density on surgery specimen noted a significant correlation. Vascular index is considered a worse indicator for evaluating of lymphatic metastases and venous micro invasion.

Although neoangiogenesis is an important step in tumor genesis, a prerequisite for tumor progression, is not the only factor that determines recurrence and metastasis. Evaluation of micro vascular density may have errors due to heterogeneity micro vascular distribution. For this reason it is necessary to analyze multiple images and sections of tumor, in order to obtain representative measurements with a high degree of accuracy.

In colorectal cancer, neovascularization is a critical event during tumor genesis, with an early peak in malignant process (Pavlopoulos, 1998; Goh, 2007; Rombouts et al., 2015).

Clinical trials performed for tumoral staging correlates high intensity of tumor genesis with tumor aggressiveness, a high microvascular density having a predictive role in development of metastases (Liu et al., 2016; Bird et al., 2018).

In T2 transversal section, the radiologic anatomy of the rectum shows the rectal lumen followed by musculara propria (which is formed sometimes from two distinct layers: internal and longitudinal), then the tunica submucosa, with high MRI intensity and muscularis mucosae, with low MRI intensity, wich delimit the rectal lumen. The muscular layer have irregular grooves steering, but uniform in size, because of the blood and lymphatic vessels which penetrate the rectal wall.

Around the musculara propria, with low MRI intensity, we can see an area of high MRI signal intensity which belongs to perirectal fat . This adipose tissue contains blood vessels with reduced MRI intensity, blood vessels and lymphatic nodes and also conjunctive septa.

Finaly, we observe another zone with reduced MRI signal, which surround the rectum and the perirectal fat. This is mesorectal fascia. Any changes of this anatomic and radiological issues should be susspected and investigated like presenting paraneoplastic origins.

The blood and lymphatic vessels have a rich density to their home area (posterior and mesorectal) which decresease as we head towards anterior. This distribution is consistent with vascular predisposition of forming a rectal cancers, especially on the rear wall or possibly on the side. Evolution to anterior of a rectal tumor is the second step in neoplastic evolution and is influenced by the anatomy of the pelvis. This occurs because of two aspects:

1. the universal law of bilateral symmetry which applies for the median unpaired organs;

2. the distance from the center of the tumor which have been performed various types of sections (3 or 5 mm).

MRI assessment contains a number of protocols which must be individualized according to need of information that are intended to be purchased, to quality of clinical and paraclinical information on the cases, and tolocal conditions. If high-quality images are needed, about the relationship of tumor to nearby organs, a MRI 2D T2 protocol with sections obtained in sagittal , axial and oblique should be used (Harris et al., 2016; Kishan et al., 2017; Lee et al., 2017; Karagkounis et al., 2018; van Ramshorst et al., 2018).

In case of obtaining inconclusive images, to better highlight both the caliber and rectal coating layer it can be used the rectal administration of ultrasound gels or contrast agents. This maneuver allows more accurate delineation of tumor poles, reduce the potential artifacts and allow a better assessment of synchronous tumors (Slater, 2006; Jeong et al., 2014).

The establishement of a normal vascular pattern of rectum was optained by comparison of angio MRI results of patients who do not show degenerative lesions at the level of this organ to those obtained by MRI with contrast in neoplasia and those obtained by arteriography performed on pieces of resection.

Major problems arise in a correct preoperative differentiating of a tumoral stage 2 by tumoral stage 3. This is a critical issue because the rectal tumor, starting with stage 3, it requires most often a preoperative radio-chemotherapy or even during surgery (Sebag-Montefiore, 2009; Sauer, 2012; Alberda et al., 2014; Holman et al., 2017). However, recent studies (Al-Sukhni, 2013) question the correctness of the assessment prognostic and therapeutic value of colorectal cancers using only information provided by MRI. For this, a group of researchers (MERCURY, 2007; Taylor, 2008; Bhangu et al., 2014), developed a local method for staging colorectal cancers corroborating investigations using MRI to determine the extramural venous invasion using the EMVI score, which envisages the histology of the tumor cells in the own vascular endothelium.

The intramural and extramural invasion of blood vessels by a rectal tumor is an important score for prognosis in evaluating patients being correlated with histologic demonstration on vascular resection specimen. The extramural vascular invasion corresponding to EMVI score 3,4 will be stage T3,T4 (Smith, 2008; Troja et al., 2015; Fleshman et al., 2015).

Venous invasion of colorectal cancer leads to the presence in blood circulation of tumor cells, which goes in the portal circulation, which results in the appearance of distant metastasis by spreading hematogenous (Krasna, 1988; Talbot, 1980; Bokkerinket al., 2015; Fleshman et al., 2018).

On the same material we performed studies on tumoral microvessels, by immunohistochemistry and quantitative microanatomic methods, which support the significant increase of tumoral microvascular density with increasing of histological degree, with an inverse proportion with differentiation degree. In addition, we noted a variation of intratumoral vascular density depending on the degree of differentiation. Interpretation of our results in terms of literature data allows us to consider that neoangiogenesis remains an independent predictive and prognostic factor, that should be considered in determining the treatment of patients with colorectal cancer.

The use of medical imaging techniques in assessing neovascularization processes is also a criterion for verifying the specificity of an immunohistochemical marker or even the connection between MVD and VEGF receptors (Henry, 2010; Stevenson et al., 2018).

Blood and lymphatic vessels have a rich density to their area of origin (posterior, mesorectal), and this decreases as we move forward, this vascular distribution being consistent with the predisposition for the formation of a rectal neoplasia, especially on the posterior wall or, possibly, the lateral ones. The anterior evolution of a rectal formation is a second step in the evolution of these tumors and is dictated by the pelvis's conformation.

As we said before, the quantification of the tumoral microvascular density is considered to be an indicator for the most of the malignant neoplasms, although sometimes the results are contradictory. In addition, the angiogenesis is heterogeneous in the same tumor (Wang, 1993; Costello, 1995) and the microvascular density assessment/evaluation is less useful in achieving new data regarding architectural complexity defined by the degree of the ramification, irregularity and tortuosity (Baish, 2000). The malignant tumors have a high vascular complexity compared to the adjacent normal vasculature; the vessels are getting chaotic or random branches (Baish, 2000; Sabo, 2001; Bonjer et al., 2015).

Colorectal and superior rectal carcinomas have a better oncologic prognosis, because arteriography showed metastasis tumor invasion only in the advanced cases; on the other hand, middle rectal tumors and especially inferior ones have a truly vascular pedicle which shows up from the early phases.

EMVI score, significantly improves the accuracy of determining a proper oncological treatment after surgery. This study raises serious questions over the current staging of colorectal cancers because changes in caliber and trajectory of rectal vessels appear early. Starting with EMVI2 score, the anatomic radiological changes which occurs seems to be caused by metastatic dissemination processes and not just by the neovasculogenesis.

Although the CD34 panendotelial marker does not indicate the angiogenesis intensity because it marks both neoformed and normal vessels, highlighting the state of vascular endothelium in colorectal carcinoma, it is considered that quantization of microvessels density (MVD) could be useful in assessing tumors's aggression. CD34 over expression was correlated with unfavorable prognosis, transition from localized form to metastatic disease and postoperative recurrence in patients with colorectal neoplasm. The anatomic-imaging staging of colorectal cancers using the EMVI score is very close to the accuracy of colonoscopic diagnosis.

The current tendencies to minimally invasive techniques, but with very high accuracy, are achieved by the correct, multidisciplinary interpretation of aspects related to paraneoplastic vascular invasion. EMVI scores are correlated with those in the classic TNM G2 and G3 stages. Concomitant assessment of patients using the CD34 marker and the EMVI score corrects the shortcomings of both methods and provides a set of important information for both the surgeon and the oncologist.

Behavior of lower rectal tumors towards lymphatic vessels in proximity is different; the fact that they have a sanguine pedicle makes their metastasis occur in this direction; in these circumstances, pelvic lymphadenopathies appear much later. In the studied cases, middle rectal vessels are more affected by the tumoral process then lower rectal vessels.

Clinical, surgical and imaging observations support the presence of a pelvic vascular paraneoplastic syndrome, kind as arterial, venous or lymphatic; the inferior rectal tumors pediculised arteries to their base, the middle and the inferior rectal tumors give inflammatory phenomena, like vasculitis or perivasculitis, especially on the path veins.

Any paraneoplasic vascular change on both rectal and pelvic vessels represents the guiding criteria of oncological surgery, being in accordance with surgical technique that is dictated by it.

Our results obtained by using the quantification of the tumoral angiogenesis via those methods could group the patients after the most useful type of treatment and also re-orientate their perspective management for those with low microvascular density. However we believe that the antiangiogenetic therapy requires individually customizing.

1.2.4. Conclusions

Our studies demonstrated the progressive decrease in the neoformation vessels lengthand the area of maximum neovascular density. This changes appeared in the tumors located in the superior, middle and inferior rectum.

EMVI score depends on the tumor location: superior rectal location has a transversal but also caudal invasion; medium rectal location has a transversal, but also a vertical invasion; inferior rectal location has vertical but also transversal invasion. Atypical invasion directions may suggest the presence of synchronous tumors or one extremely aggressive. Studies are required on extensive groups of patients in order to make the EMVI score a routine method in the diagnosis and prognosis of colorectal neoplasia.

Ki-67, marker of cell proliferation is associated with a low prognosis in colorectal cancer patients. Its expression is increased in patients with Dukes C or D colorectal cancer versus those in stage A or B. Increased expression of the Ki-67 marker is generally associated with a low prognosis of survival, especially in undifferentiated or low differentiated cases of colorectal carcinomas. The Ki-67 along with other clinical-pathologic markers could be useful in the prognosis of patients with colorectal cancer.

In the studied cases it can be seen a pelvic vascular paraneoplastic syndrome both of arterial, venous and lymphatic origin.The number of vascular nucleuses of a lower rectal tumor is proportional to intraoperative and postoperative bleeding risks, as well as to the possibility of developing postoperative abscesses.

1.3. Anatomo-surgical and imagistic aspects in cervical tumors

Staging of gynecological cancers is based on surgical pathological findings, with the exception of cervical cancer, which is clinically diagnosed, as it is stipulated by International Federation of Gynecology and Obstetricians (FIGO). This is currently the most commonly used staging system (Pecorelli, 2009).

MRI is the most accurate method of examining the female pelvis, being considered superior to computed tomography in tumor extension evaluation and capable of appreciating ganglionic lymphatic system. Nuclear magnetic resonance provides the most accurate data on cancer extensibility and allows perfect anatomical delimitation, eventually enhanced by MRI

contrast products (Bourgioti, 2016). Nuclear magnetic resonance investigation may be associated with contrast agents. The evolution of these substances over time is critical, there are nowadays as few adverse effects and are eliminated from the body by the kidneys within 90 minutes.

Among the most popular contrast agents are those called paramagnetic and based on several ions with one or more free electrons. In the category of these most commonly used ions is gadolinium, subject to chelation because it induces the most powerful relaxing effect (Santoni, 1987). Also, T1 sections are commonly used because there is an increase in signal intensity and a positive contrast signal with the magnetic dipole of neighboring protons.

The aim of this study is to investigate the possibility of using these substances in early diagnosis and cervical cancer staging (Khemthongcharoen, 2014), following a protocol that correlates the extramural blood vessel aspects (EMVI score) with the TNM staging. This protocol has been previously used by us, but only in patients diagnosed with colorectal cancer.

1.3.1. Materials şi methods

The study was performed in a group of 17 patients admitted to the ”Cuza Vodă” Clinical Obstetrics and Gynecology Hospital, diagnosed with cervical cancer with various localizations. Patients were investigated by: clinical examination, cervico-vaginal test, colposcopy with biopsy, anatomopathological examination, CT and MRI in order to perform a preoperative imaging evaluation and to provide information about tumoral location, tumoral staging with depth of invasion, and their correspondence to fascies, adjacent organs and blood and lymphatic vessels.

The MRI exam was performed on a 0.23T (low field) PICKER OUTLOOK PROVIEW, accessible especially to claustrophobic, anxious and obese patients. The sequences used were T2TSE, T1TSE native and postcontrast, spectral suppression with inversion recovery (STIR) to suppress the signal from the adipose layer.

T2 sequences were purchased in at least 2 planes - axial and sagittal (most useful for cervical pathology), optionally coronal, with small FOV (field of view), respectively increased resolution for evaluation of uterus, cervix, vagina and parametryum . Paraaxial T2 sequence (perpendicular to the long axis of the cervix) is preferred especially for the evaluation of the parametryum invasion.

The T1 axially-sequence with large FOV were used for scanning the entire pelvis, with particular attention to the detection of low paraaortic and inguinal adenopathies, respectively the assessment of bone structures in the examination field for the detection of possible secondary determinations.The examination protocol provides sections in multiple sequences - T2 TSE, T1 TSE, with sections of 1.5 mm thickness and 2 mm interval. The STIR examination involved sections of 5 mm and 5 mm thick.

Searching for tumor extension in the vagina (best appreciated on axial and sagittal sections), it were performed sections in sequences - T2 and T1 postcontrast (differentiates stage IIA from IIB). The parametryum for extracervical tumor extension, T1 sequences without fat saturation and T2 and T1 postcontrast with fat saturation are inspected. They are very sensitive for the extent of disease - stage III B parametryum.

We followed the imprecisely delimited sequences that infiltrate the adipose tissue of parametryum, respectively the signal intensity of invaded parameters of fat. MRI appreciates the status of ureters in axial and/or sagittal sections in T2 sequences for abnormal dilation following neoplastic invasion - stage III B.

The MRI criteria used for bladder or rectal invasion were:

- focal absence of the perivezical or perirectal lipomatous plane;
- the presence of an endoluminal tumor mass in continuity with the primary cervical tumor.

The nodular aspect of the rectal or bladder wall as well as the demonstration of a fistula tract were also followed.

1.3.2. Results

The inspection of the pelvic lateral walls is done not only to indicate the direct tumour extension but also to indicate adenopathies. It is essential to highlight the invasion of parametryum and the damage of the fibrous stroma (Figure 1.30.).

MRI T2 sections were used to demonstrate a number of prognostic factors for local disease recurrence and survival rate in patients with cervical cancer. These cases have initially recomandationsfor histopathological studies. These prognostic factors include: the tumoral extension to the lateral resection margin, the depth of the extramural tumoral extension and the presence of the extramural venous invasion (Figure 1.31.).

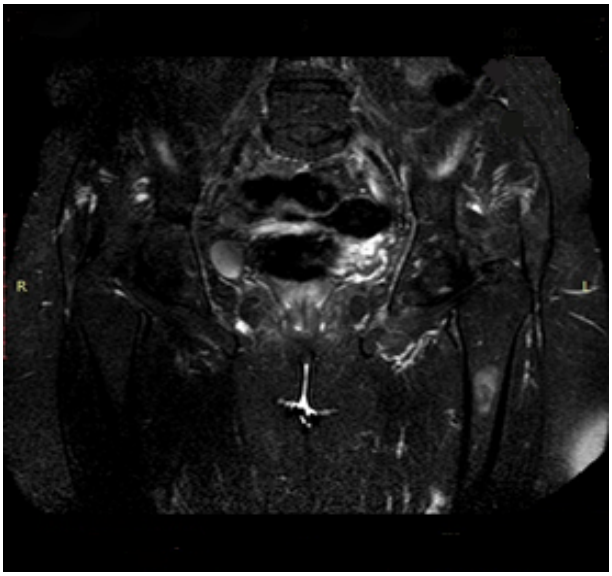

Figure 1.30. Long-distance tortuosities and nodosities specific to IV EMVI score in stage IIB of cervical neoplasm

Extramural vascular invasion (EMVI) is defined as the presence of tumoral cells in the vasculature beyond the *muscularis propria*. It is produced by small tumors that penetrate deeper and beyond the parametryum and is known to be a poor marker for survival and recurrence of the disease. On MRI images, EMVI score gives a serpentine appearance of tumor extension within a vascular structure (Figure 1.32.).

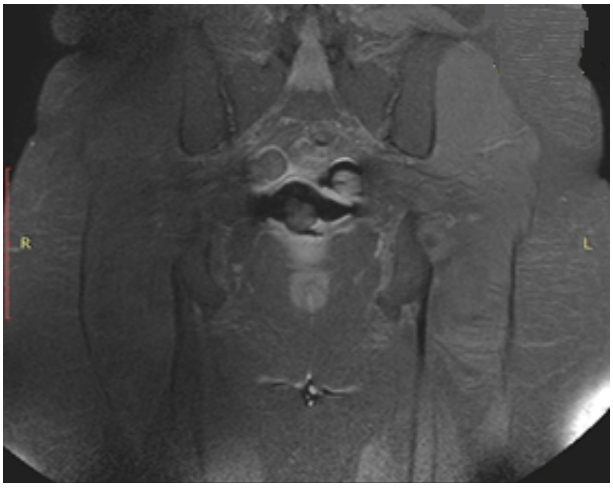

Figure 1.31. Tumor formed with an anular effect at the level of homolateral intramural vessels

Figure 1.32. Sagittal MRI, EMVI II-III score with intramural vessels in cervical neoplasm in TNM stage II

MRI correlation with histopathological evaluation gives a much more accurate estimate of EMVI scoring.MRI has the advantage of in vivo demonstration of vascular anatomy and therefore, tumor invasion can be immediately identified. For these reasons, the evaluation of the presence or absence of EMVI scoring should be a routine in our hospitals. Stage I EMVI can be diagnosed if correlated with the histopathological examination (Figure 1.33.).

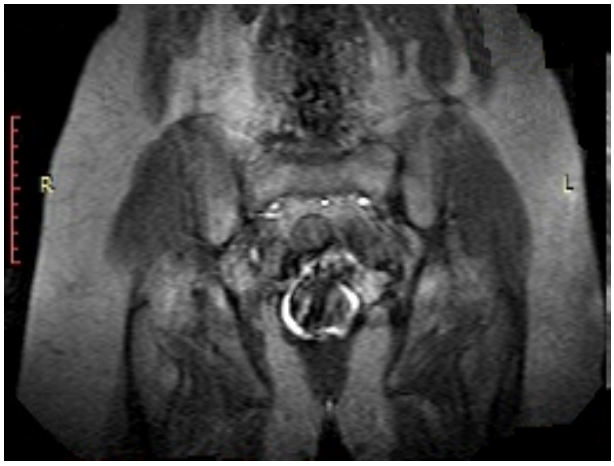

Figure 1.33. Transverse IRM, EMVI I score in cervical neoplasm in TNM stage I, intramural vessels

1.3.3. Discussions

Extrauterine venous invasion can also have a role in evaluation of preoperative risk, reconsidering decisions about chemotherapy or intensifying neoadjuvant treatment.A good EMVI regression of fibrosis (> 50%) is associated with a significantly better survival rate, independent of final staging.MRI is more accurate associated with EMVI identification for large vessels compared to small calibers ones. Although EMVI identification of small vessels is more difficult to achieve from a radiological and histopathological point of view, this would seem to have reduced clinical consequences.

Preoperative tumor staging leads to an individualized, surgical and oncological therapeutic protocol.

Differentiation criteria for blood vessel invasion are relative and should be correlated with the TNM classification. This applies in stages I and II of evolution, while in the advanced ones the radiological signs are eloquent.

It should be noted, however, that the initial stages of tumorcan present direct and indirect changes to the blood vessels from the tumor proximity. Although direct changes refer, particularly to endothelial vein metastasis of cancer cells, tumors can alter vascular appearance by phenomena that occur at the beginning of neoangiogenesis processes.

Basically, angiogenic changes occur in the initial tumor stage, and in late stages it can even be found endovenous and lymphatic neoplasm blocks (objectified through extrinsic venous stenosis).

These signs and criteria dictate the type and opportunity for the surgery to be chosen in each case.

Parameter invasion is a very important stage in the MRI examination of cervical cancer with the evidence of thefibrous ring that is hypodense. If it is continues around the lesion, we can say there is no stromal invasion (Stage I B FIGO).

Focal disruption of the fibrous ring with direct tumor extension is a sign of invasion of the parameters (Stage II B FIGO).

Regarding*vaginal invasion*, it can be said whether there is a direct extension from the tumor to its walls or partial obliteration of vaginal sac. It is important to specify the staging of the invaded area (upper third or two-thirds lower).

The *bladder invasion* is confirmed if in T2 hyperintensive, tumor is replaced by the hypointense signal of the vesicle wall and then the deletion of the fat plane separating the cervix from the bladder. It is important to differentiate a lesion of the entire bladder wall from a limited lesion of the muscles alone (Small et al., 2017).

Rectal infiltration is confirmed if in T2 the hyperintensive signal of the tumor replaces the hypotensive signal of the rectal wall and after deletion of the fat plane separating the cervix by the rectum. By injecting the contrast substance, we get a better view of the structures (Bhatla et al., 2018; Hecking et al., 2018).

Ureteral infiltration is confirmed by direct tumor extension or ureteral compression by them. The urographic sequences in T2 hyperpondery allow for the overall visualization of the dilated ureter and accurately determine the location of the obstruction.

Pelvis wall infiltration is defined by the presence of the tumor less than 3 mm from the wall. This is actually formed by levator ani muscles, internal obturators and pyriforms. The direct invasion is definite by the disappearance of the paramuscular fat plane, the presence of a T2 signal in the muscle, or the intensification after contrast substance injection into the muscle in direct continuity with the tumor (Duska et al., 2017; Sakuragi, 2015).

The *lymphatic*, pelvic or abdominal invasion is based on the following criteria: oval-shaped nodes whose large axis is over 1cm or rounded-shape nodes with a diameter of more than 8mm. From a morphological point of view, the following signs are investigated:

- irregularity of the nodecontour;
- thin fat perganglionic composition;
- necrosis plates more or less stretched;
- heterogeneous aspect of the lymph nodes.

Cervical cancer has an intermediate signal in T2 (hyponormal to normal cervical mucosa and hypernormal to fibromuscular internal stroma). Crucial for diagnosis is the appreciation of irrigation of the cervical fibrous stroma (Horn, 2007). The low signal differentiates stage IB from IIB (Kurman, 2002). In axial and sagittal T2 and T1 sections, with contrast paramagnetic agents, we followed the occupation and invasion of the tissue planes that separate the cervix from the bladder or rectum - stage IVA (Hricak, 2014; Carmichael, 1984).

The accuracy of MRI staging is between 76-92% (Cantor, 2008; Lester, 2006).

The normal uterine cervix is perforated by numerous small venules that appear on MRI-T2 sections with a intensity signal from weak to intermediate. Extramural venous invasion is recognized on MRI-T2 sections by the expansion and irregularity of venules adjacent to the primary cervical tumor due to contiguous tumor extension. The involved vein generally appears by intermediate intensity signal and loss of normal vascular flow.

The lymphatic vessels accompanying the blood vessels are much smaller than these and therefore can not be viewed on the MRI -T2 sections. However, there is evidence from specialized literature that tumoral invasion of extramural veins acts as a surrogate marker for lymphatictumoralpermeability. Therefore, patients with apparent extramural venous invasion

at MRI have theoretically a greater risk and incidence of lymphatic invasion (Pfaendler et al., 2015; Katepratoom et al., 2014).

From a practical point of view, the recognition of MRI features that are associated with increased risk of lymphatic invasion may alert the treating physician to enhance the assessment of lymphatic status and also to increase the arguments for neoadjuvant radiochimioterapies in order to reduce the recurrence of local invasion.

MRI may be superior to histopathological analysis of resection specimens in EMVI identification, especially if surgical technique is limited (Messenger, 2012; Viswanathan et al., 2014).

Using preoperative acquired images we applied diagnose staging by using the EMVI score. We correlated our results with the usual staging of TNM.The most important anatomical surgical aspects are the sacral rectal genital pubic fibrous fascicles and the relationships with the surrounding organs.

Histopathologically, extramural venous invasion demonstrated that cervical cancer was associated with a lower survival rate and increased risk of lymphatic invasion and remote metastases.

An EMVI score higher than 2 in MRI T2 sections has a high sensitivity and specificity for venous invasion of more than 3 mm in diameter, as it was histopathologically demonstrated. However, the EMVI score has a moderate sensitivity in predicting lymphatic nodes involvement.

Weaknesses identified in the protocol:

- *sensitivity of the method in the early stages*
- *mode of differentiation of pre-operative T2 and T3 stages (which requires the need for neoadjuvant therapy)*
- *establishing a new protocol by integrating existing knowledge with the aspects obtained by angiographies performed on the resection specimens, anatomopathological results, immunohistochemical preparations and specific neurohormonal markers.*

Criteria for improving the EMVI Score:

- *the appearance of the margins of the tumor - the tumoral invasion in the small veins can produce nodosities of their wall, distinct from desmoplasia*
- *localization of the tumor against large vessels - the presence of the tumoral signal in the vascular structures*
- *calibrate affected vessels - these increase in volume, the signal inside them is a medium gray*
- *the margin of the blood vessels - the tumor can be expanded inside the vessels and can give a look from smooth to irregular or nodular*

Following the correlation of the investigations carried out in the cervical neoplasia with the pre-existing rectal studies, in the case of uterine cervix, approx. 46% of the cases studied had specificity and diagnostic accuracy and prognosis. For the most part, these cases are in advanced stages - IIB and IIIA and B. The data obtained in the study could reveal aspects of extramural invasion in Stages IIB and IIIA and IIIB corresponding to confirmed

data in colorectal cancer, which promises the accuracy of this new method in cervical neoplasia.

1.3.4. Conclusions

MRI is a widely used method of investigation, with very good acuity in most cases of colorectal neoplasms that might be attempted to be extended to cervical cancers. The current tendencies towards minimally invasive techniques but with very high accuracy are achieved by the correct multidisciplinary interpretation of the aspects related to the paraneoplastic vascular invasion. The advanced stages of the EMVI score correlates with those of the classic TNM stadium. The tumoral invasion of the extramural veins surrounding the primary lesion in cervical cancer acts as a surrogate marker for lymphatic permeation of the tumor. Therefore, patients with obvious venous extrauterine invasion at MRI have theoretically a greater risk and incidence of lymphatic invasion. EMVI scoring system could be used in other tumour staging, such as cervical, endometrial or vesicale. Each outer wall of the pelvic organs are pierced by thin veins which and their aspect is under influence of visceral functionality.

1.4. Further enhancements

The practical implications of these studies go from a common point - the development of radiological criteria to identify vascular abnormalities that may be correlated with the development of adjacent neoplasia. In this respect, the vascular aspects get different practical connotations depending on the type of affected blood vessel. The number of blood vessels increases and they are organized in local networks, near the tumor. The venous vessels, in turn, suffer changes in trajectory, caliber and appearance without forming new ones.

Any atypical disposition of blood vessels may be considered an indirect sign of pelvic neoplasia.

2. ADVANCES IN FASCIAL STRUCTURES OF THE FACE

Brief history of superficial musculo-aponeurotic system concept

The superficial musculo-aponeurotic system (SMAS) was described in 1974 by Paul Tessier (Leturneau et al., 1988) as an indispensable entity of plastic surgery. Then it was identified through dissection (Mitz and Peyronie, 1976), beeing a guiding structure for the plastic surgeon.

There is a great variability in the histological aspect of SMAS in different facial regions of the same individual, but also between the same region in different individuals (Jost and Levert, 1984; Pensler et al, 1985; Barton, 1992; Mitz, 1992; Gosain et al. 1993). Because of this variability, histological identification must pursue continuity with known reference structures, such as platysma.

Anatomists (Hollinshead and Rosse, 1985; Jacob, 1996; Lippert, 2000; Berkovitz and Holland, 2002; Gosling, 2002; Benninghoff – Dreckhahn, 2003) are not in consensus on describing this structure.

Subsequently, there have been several studies demonstrating the presence of SMAS in different regions. Among those who have strongly promoted this concept are Mitz and Peyronie that define SMAS as a fibromuscular network between facial muscles and dermis, which covers the facial nerve and its branches (Mitz and Peyronie, 1976).

Approximately five years after the new concept was introduced, a series of applications in surgery, such as plastic surgery, oro-maxillo-facial surgery, head and neck surgery, neurosurgery and cosmetic surgery, began to appear.

At the same time, this theory has many opponents who deny the existence of SMAS (Jost and Levet, 1984).

Thaller et al. (1990), after they have done fresh body dissections, topographic histology and comparative anatomy, revealed the presence of SMAS as a distinct fibromuscular layer, consisting of the platysma muscle, the parotid fascia and the fibromuscular layer covering the cheek.

Anatomical studies by using tissue plasticization (Gardeto et al., 2003) identified SMAS in the parotid region where it is thick and attached to the parotid fascial sheath. In the mouth region it is thin, can not be dissected, and in other regions, such as the nasal region, did not identify it.

Besides these, a third trend of opinion promoted by Gosain et al. (1993) considers that SMAS is in continuity with platysma but has no direct muscle insertion and is not related to the temporocycipal fascia.

One thing, in turn, is recognized by most authors, namely that SMAS can be macroscopically highlighted at the parotid region, otherwise it can be observed with the microscope. It continuesto the neck with the platysma muscle fascia. There is a close concordance between the development of the fascia and that of the muscles of the cervical region. This is obvious after birth, in the adult, by the presence of a morpho-functional link between the two layers.

In view of these considerations, a number of explanations are needed regarding the notion of SMAS of the face, its origin and embryogenesis, and finally the systematization of some anatomical conclusions adapted to the new techniques of plastic and reconstructive surgery.

The soft tissues of the face are supported in a normal anatomical position by a series of ligaments extending from deeply to the deep dermis (Stuzin et al., 1992). A continuous, organized, fibrous network, specific to nasolabial and upper lip, frontal, parotid, zigomatic and infraorbital regions (Ghassemi et al., 2003), is organized.

Most studies confirm the observations of Mitz (1992) and believe that SMAS belongs to the cervical-cephalic fascia and is tensioned by:

- *superior* - the superficial temporal muscle, frontal muscle and orbicularis oculli muscles,
- *inferior* - platysma muscle,
- *posterior* - adhere to tragus and mastoid process.

SMAS covers the muscles of the facial expressions that adhere to it and comes in close, deep relation with the facial nerve.

The anatomical relationships between the facial layers of the face can be systematized as follows:

1. the *superficial face fascia* covers the muscles of the facial expression (platysma, orbicularis oculli, major and minor zygomatic muscles);
2. the *deep facial fascia* represents the continuation of the neck, its importance lies in the fact that the branches of the facial nerve in the cheek are deeply found in this deep fascial layer;

Two types of relations can be established between the two fascicles:

- In some regions of the face the two layers are separated by an areolar plane,
- In other regions of the face, the superficial and deep layer is intimately adherent to one another through dense fibrous tissue.

At the head and neck level, the superficial fascia does not have a role of separation between superficial muscles and skin regions, but a role of cohesion, linking them to the skin.

The implications of SMAS are found in other branches of medicine, not just in surgery (see the rate of propagation of an infection from the cervical region to the face).

Through the personal studies underlying this chapter, I propose to explain the surgical techniques based on the unique blanket concept - SMAS - and its applications. At the same time I will show that some surgical techniques support the existence of SMAS in the head and neck.

Cosmetic surgery today would not be possible without SMAS. Old cosmetic surgery techniques that did not take into account (or were not known then) SMAS could leave mutilating scars on the patients.

At the same time, SMAS brings a new and powerful light to the cheek region surgery, which was previously considered a true jungle where not many surgeons would venture. It provides a logical explanation for the skin fixation and traction vectors.

The face, the expression of the phenotype of each of us, is part of our social life and a mirror of the soul. It is characteristic at the individual level and yet it is based on common anatomical structures. All of these structures have not yet been fully elucidated even from a macroanatomic point of view, being even considered a long time surgically inoperable. It is a complex structure, both anatomically and functionally, requiring a high degree of knowledge and understanding.

Mitz and Peyronie (1976) strongly promoted the concept and defined the SMAS as a fibromuscular network located between the facial muscles and the deep dermis, which covers the facial nerve and its branches. In support of this theory, there are a number of issues, such as:

- explains the functional mechanisms of facial expression muscles;
- the embryology of the superficial face and neck planes shows that the development of the muscles of facial expression, superficial and profound adipose tissue, facial nerve and parotid gland are the result of divergent movements and migratory confluences, which eventually led to the anatomical definition of a superficial blanket, unique on the face;
- explains the anatomic - functional mechanisms of the "ageing face";
- today's cosmetic surgery would not be possible without SMAS.

SMAS represents the fascia of the facial expression muscles and continues inferiorlywith platysma muscle, superiorly, above the zygomatic arch with a similar layer, temporoparietal or temporal superficial fascia and laterally covers the parotideomassetric fascia (Mitz and Peyronie, 1976 ; Furnas, 1989; Tessier, 1989 ; Stuzin, 1992). The superficial layer of the deep fascia bifurcates and covers the parotid gland, the superficial lamella being briefly referred to as the Parotid Fascia. The branches of the facial nerve are deeply found in this layer, which continues above the masseter muscle under the name of parotideomaseteric fascia.

SMAS establishes links with both the skin of the region and the muscles of facial expressions, deep fascia and subjacent neurovascular elements (Benedetto et al., 2005). It is deeper than the subcutaneous adipose layer, although it is laterally adherent to the parotid fascia, where it is known as immobile SMAS. Prior to the parotid gland, there is a lax areolar plane under SMAS, which allows the mobilization of the anterior region of SMAS (Mitz and Peyronie, 1976; Stuzin, 1992). This layer is crossed by ligaments dirrected to mucles from abov, ligaments that may have mobility in some regions (on the side of the eyebrows or in the inferior-lateral part of the orbit) or may be fixed and tense in others such as the medial portion of the mandible or of the orbit (Furnas, 1989; Stuzin, 1992; Ghavami et al., 2008).

Controversies that still persist in the literature on SMAS behavior in various areas of the face and neck, the different degree of mobility and the existence of fixation formations determined me to deepen this anatomical structure by trying to produce macroscopic, mesoscopic, microanatomic and imagistic evidence regarding the existence of this structure in different regions of the face, the particular regional characters, correlating them with their importance for the maxillofacial and plastic surgeons, but also for the orthodontic surgery.

This research direction has been materialized by publishing the following articles:

1. Hînganu D, Scutariu MM, Hînganu MV. The existence of labial SMAS — Anatomical, imaging and histological study. Ann Anat 2018; 218: 271-275.
2. Hinganu D, Stan CI, Taranu T, Hinganu MV. The anatomical and functional characteristics of parotid fascia. Rom J Morphol Embryol 2017; 58(4): 1327-1331.
3. Hînganu D, Stan CI, Ciupilan C, Hînganu MV. Anatomical considerations on the masseteric fascia and superficial muscular aponeurotic system. Rom J Morphol Embryol 2018; 59(2):513–516.

2.1 Introduction and conceptual backgound

Within the complex cervical and facial structures, the superficial fascia is still a subject of research and dispute between researchers.

The main description of SMAS was made by Mitz and Peyronie in parotid and cheek regions, which stimulated many surgeons to perform layer by later dissection of the regions of the face. The most complete descriptions are made in the cheek (Bosse, 1987). It is

admitted that the fascial layer that cover parotid gland and its capsule is constituted by the parotidomasseteric part of the SMAS and skin (Gardetto, 2003).

Gola (2005) considers that this extension to all face regions is abusive without sufficient data.Delmar (1994) considers SMAS as a strictly surgical superficial anatomical structure derived from primitive platyms and risorius muscles, and Thalller et al., considers SMAS a distinct fibromuscular layer consisting of platysma muscle, parotid facia and fibromuscular layer of the cheek (Gola, 2005; Delmar et al., 1994).

Gosain et al. (1993) describe for SMAS some anatomical features, which we present schematically:

1. although SMAS is intimately applied to the superficial surface of the parotid, a thin but distinct parotid fascia is identified between the gland and SMAS.
2. the SMAS terminates 1 cm below the zygomatic arc and does not continue with the temporal fascia.
3. in the cheek, SMAS, continue with the orbicularis oculli muscle of the lower eyelid eye.

There is a great variability in the histological aspect of SMAS in different facial regions, describing individual and regional peculiarities to the same person. Because of the large microanatomic variability, SMAS can be histologically identified in the continuation of reference structures such as platysma. It is characterized by extreme variability in thickness, from one individual to another, and in the same individual, on right or left (Gosain, 1993).

The term SMAS defines a musculoskeletal sheath that continues the frontal muscle to the platysma and is "a facial muscle contraction amplifier". It acts as a distributor of muscle contraction to the skin: each muscle contraction follows a preferential direction in the network. An infinite number of actions are possible because, on the one hand, SMAS conveys the contraction of the muscles along the parallel network to the skin plane, and on the other hand transmits the resulting effect in a direction perpendicular to the skin through fibrous expansions from SMAS towards dermis. It presents two parts in continuity with each other: cervicofacial, essentially aponeurotic and cervical, essentially muscular (Mitz, 1976; Bosse, 1987).

- ***cervicofacial SMAS*** consists of two parts:

1. *parotidomasseteric* SMAS, well personalized, may contain some muscle fibers. Cover parotid lodge and masseter muscle. Riolsn in Le Double (1897) signaled the " portio musculi cutanei supra parotidem ad aurm ascendentis", a part of the platysma muscles that ascend to the ear, passing over the parotide. It is fixed to the auricular cartilage and establishes deep adhesions with the parotid capsule. In the subangulomandibular and mastoid region it adheres to the superficial cervical aponeurosis that covers the sterocleidomastoideus muscle.
2. *jugal SMAS*, thin and continuous, difficult to dissect, gradually thinning from the posterior to the anterior and not exceeding the nasolabial groove. Contains the risorius muscle that is paracomisually directed to the modiolus, and pulls it back in the smile. This muscle develops in SMAS thickness before masseter aponeurosis, but without inserting on it.

- ***cervical SMAS***

At the lower edge of the mandible, SMAS cervical is confused with the platysma muscles, prolonging cervicofacial SMAS. The muscle, located on the side of the neck, consists of two fascicles with different actions:

- *lateral fascicles (*pars buccalis), the italic S-shaped slides on the posterior part of the basilar mandibular margin and over the mandible angle to attach the comissure,
- *medial fascicle* (pars mentalis).

All these data on the existence of a single cervico-facial musculoaponeurotic cover provide the background for modern plastic and reconstructive surgery. At the same time, they provide a solid support in the precise understanding of functional anatomy regarding superficial cervico-facial layers and physiological mechanisms that compete in the fight against the phenomena of aging face.

2.2. Anatomical approach on the parotid masseteric fascia and SMAS

It is important to understand the disposal of subcutaneous layers in different regions of the face because of their implications in various surgical fields. The superficial muscular aponeurotic system (SMAS) of the face is a guiding structure for the plastic surgeon, especially in the masseteric region.

In view of these considerations, a number of clarifications are needed regarding the notion of SMAS of the face, its origin and embryogenesis, eventually systematizing some anatomical conclusions adapted to the new techniques of plastic and repair surgery (Mitz, 1976; Bosse, 1987).

There is a great variability in the histological aspect of SMAS in different facial regions in the same individual, but also between the same region in different individuals.

SMAS presents as a distinct fibromuscular layer, consisting of the platysma muscle, the parotid fascia and the fibromuscular layer covering the cheek (Gardetto, 2003). A continuous, organized, fibrous mesh, specific to the nasolabial and upper lip, frontal, parotid, zygomatic and infraorbital regions, is achieved (Gola, 2005).

The parotid fascia is forming a capsule around the gland, together with the masseteric fascia. It sheaths the parotid gland, its excretory duct and the branches of the facial nerve as well.

Commonly is considered that parotid fascia proceeds of the superficial layer of the deep cervical fascia, which splits to cover the gland (Mitz, 1976). It is named parotid fascia because it is related to the lateral side of capsule. The fascia itself is made of two layers: lamina superficialis that runs upwards to be continued by the temporal fascia and lateral by the masseteric fascia, lamina profunda that covers the stylohyoid, the styloglossus and stylopharyngeus muscles.

The superficial layer attaches to the zygomatic arch and to the mandibular body. The aim of this study is to objectify the origin of the superficial parotid fascia, which is considered to be a duplication of the deep fascia, by analyzing its quantitative and qualitative anatomy. Plastic surgeons (Bosse, 1987) consider that the superficial fascias of the mentonian, parotidian and cervical regions are interconnected. The entire connective tissue that makes this connection together with the skeletal muscles attached to it is nowadays considered to form a muscular aponeurotic cervico-facial unit (Gardetto, 2003).

The importance of our study derives from the practical utility of the superficial muscular aponeurotic system (SMAS) concept, which is continues also at parotidian level.

This finding allows surgical techniques to minimize postoperative incidents. The main surgical approach to parotid tumor formations based on the SMAS concept is extracapsular lumpectomy with SMAS flap (Gola, 2005; Tamplen, 2016; Berry, 2010).

Basically, by preserving a flap of SMAS in parotid interventions, it can be reduced the risk of parotid Frey syndrome (gustatory sweating), and also minimizes the risk of postoperative infection and allows reconstruction of the parotid lodge (Khan, 2014; Bonanno, 1992). This is possible by maintaining characteristics of the facial SMAS at this level: it forms fascial tunnels for arteries and nerves and continues in neighboring regions. SMAS transmit, distribute and amplify the activity of all facial muscles (Gianonne, 2008). The existence of SMAS at this level is not fully recognized by researchers (Dell'Avarsana, 2015; Lee, 2017).

Indirect has long been used in plastic surgery techniques, but maxillofacial surgery partially adopts this concept and not from long time ago (Quer, 2017; Owsley, 1983).

The aim of the study of this study is to identify the particular morphofunctional features of the SMAS in masseteric and parotid regions and to show their practical significance. It also brings quantitative and qualitative evidence of the existence of SMAS at the lateral regions of the face and corresponding neurovascular elements.

2.2.1. Material and methods

The material used was represented by 12 cephalic extremities, which were meticulously dissected in the "Ion Iancu" Anatomy Institute of "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania.

Each specimen was previously preserved in formaldehyde. Dissections were performed layer by layer and, on each stage of dissection mesoscopic images were captured by using the Kaps SOM 62 operating microscope.

The conclusive aspects were acquired, examined and further processed to remark the regional stratigraphic differences. We collected parotid tissue samples, through a perpendicular incision in the skin, which went deeper until level of parotid fascia.

Simultaneously, we have conducted a study on a group of 10 patients admitted to the Clinic of Maxillofacial Surgery, "St. Spiridon" Emergency Clinical Hospital, Iaşi.

Patients who were clinically and imagistically diagnosed computed tomography (CT), magnetic resonance imaging (MRI) with parotid tumors and underwent surgical interventions for total or partial parotidectomy or in order to plan a surgical masseteric procedure.

For the qualitative microanatomical study of SMAS in the stratigraphic layers of the face, we sampled all the soft parts of the facieses of the anatomical specimens, from the skin to the bone, in the form of small blocks, with the following topography: premasseterin and masseterin.

The dissection was performed perpendicular to the surface of the epidermis in order to be able to follow the correct sequence of the plans.

The operatory fragments have been removed in order from superficial layers to deep fascia, during facial lifting and other facial interventions on these regions and compared the results with CT and MRI aspects.

The collected formalin specimens were precessed by paraffin technique and stained with special techniques for muscular and connective tissue (Verhoeff and Szekely).

Stereology was used with the standard Weibel parallel grid to quantify the percentage volumes of the main structural parietal components in the studied vessels.

Surgical interventions have allowed us segmental anatomical studies, depending on the surgical procedure, providing in vivo visualization of the fascial and muscular structures, evaluating the possibilities of dissociating the plans and appreciating their vasculature.

The collected specimens were processed by paraffin technique and stained with special techniques for muscular and connective tissue (Szekely).

2.2.2. Results

SMAS can be macroscopically seen in the *masseteric region*, otherwise it can be observed with the microscope, laterally forms the anterior sheet of the parotid fascia. Although SMAS is intimately applied to the superficial surface of the parotid, a distinct parotid fascia is identified between the gland and SMAS, which extends to the masseteric region (Figure 2.1.).

Figure 2.1. Parotid cervicofacial SMAS continues to be superior with parietotemporal fascia. Vessels are observed superficial and their branches. Dissection specimen.

The cervicofacial cutaneous muscular aponeurotic unit is separated from underlying, musculo-aponeurotic or periosticular plans by deep subcutaneous or cellular tissue, that functions as a sliding plan. If deep subcutaneous cellular tissue is reduced, SMAS adheres to the underlying plans and the skin loses part of its mobility.

The neurovascular elements in its immediate neighborhood are numerous and complex. From this point of view, the complexity of the parotid region where the facial nerve is found (it is divided into the terminal branches within the parotid gland), as well as branches

of the external carotid artery, has to be emphasized. Lesser (superficial) masseteric nerve, together with superficial masseteric artery and vein are running between SMAS and the masseteric fascia.

The branches of the facial nerve are also in the vicinity of the SMAS of this region, as well as branches of the trigeminal nerve. The facial nerve branches are considered the most variable anatomical elements, but the use of landmarks allows us to locate them accurately (Figure 2.2.).

Figure 2.2. Branches of facial nerve related to SMAS. Dissection specimen.

These nerve threads are accompanied by branches of the external carotid artery. When removing skin flaps or performing pretragal incision for facial lifting, surgeons must consider these relation of the superficial masseteric fascia (Figure 2.3.).

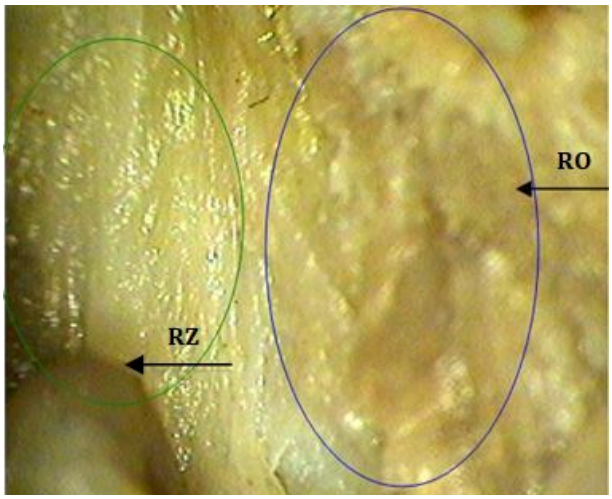

Figure 2.3. The limit zone between the zygomatic region (RZ) and oral region (RO). Mesoscopic images.

The masseteric region makes the transition to the zygomatic and temporal regions. The morphological, macro and micoscopic differences between the these regions are clear and suferficial thick masseteric fascia continues anterior superior with the zygomatic fascia and posterior superior with the temporal one.

Inferior posterior, at the level of the parotid gland, the superficial fascia gives rise to the anterior capsule of the gland.

Anterior medially it is continued by the superficial fascia of the cheek and anterior medially forms fibrous tunnels through which the branches of the facial nerve divisions pass (Figure 2.4).

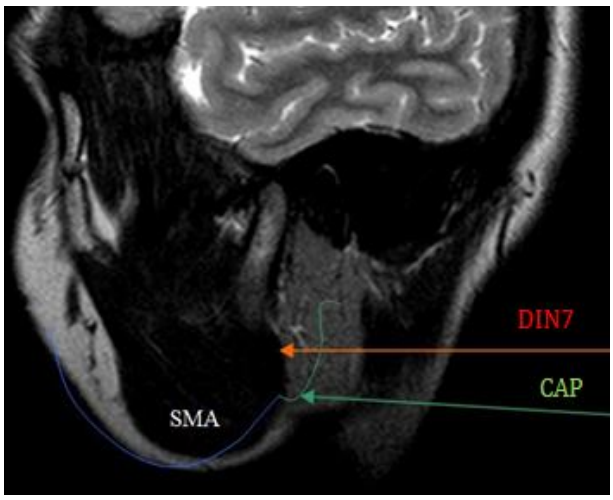

Figure 2.4. Anterior parotid capsule (CAP);
Intraparotid division of the facial nerve (DIN 7)

Superficial masseteric fascia is continued downwords by superficial parotid and cervical fasciae, where it originates from (Figure 2.5.).

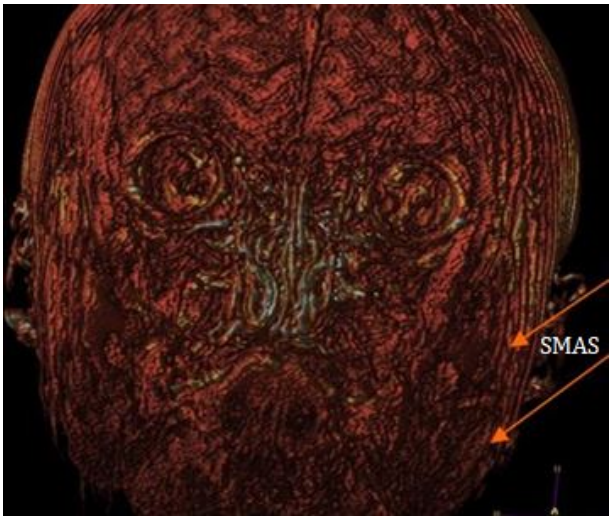

Figure 2.5. 3D coronal reconstruction that highlights
the topographical plan of SMAS from tragus to gonion

The most important features of soft tissues stratigraphy at this level are:

1. the superficial fascia (SMAS) is best represented here, consisting of dense connective tissue;
2. the superficial fascia is connected to surrounding fasciae and works as one great muscular aponeurotic complex.
3. subcutaneous adipose tissue is predominantly prefascial but its consistency and form is different from zygomatic, temporal and parotid regions.
4. the superficial fascia gives rise to the zygomatic and inferior temporal ligament
5. the superficial fascia is crossed by vascular-nervous elements.
6. superficial layers have very low mobility in the center of the region.

In conclusion, we can systematize that the lateral region of the face has as layers:

a. cutaneous, dermoepidermic,
b. subcutaneous fat,
c. superficial fascia,
d. the branches of the facial nerve,
e. deep, masteterin fascia,
f. the muscular layer, represented by the masseter muscle.

Microscopically, SMAS is well represented, with condensed colagene thick, orderly disposed, mostly longitudinal, with diminished interstitial spaces. Blood vessels meet only at the periphery of the lamine and have very small dimensions.

The distance to the deep fascia is increased, the infraSMAS fat layer being well represented with oblique orientation, the elastic fibers completely missing.

The superficial fibrous connective layer is well represented, with many fat lobes separated by connective tracts with almost vertical or slightly oblique direction. There are predominantly medium-sized collagen fibers, rare elastic fibers, almost similar with the arrangement into parotid region.

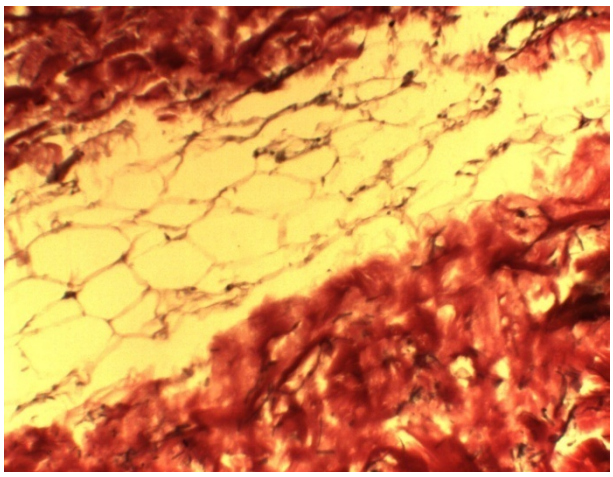

Figure 2.6. Succession of superficial layers in the parotid region, top-down: SMAS, adipose tissue infraSMAS and parotid fascia. Verhoeff staining, ob. 40x

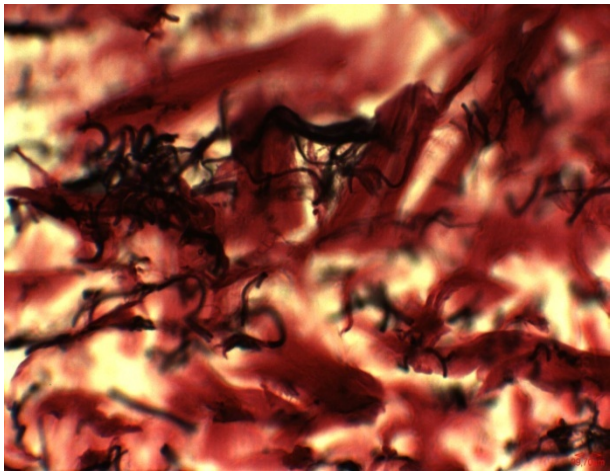

Figure 2.7. Parotid dense structure, with many collagen fibers thick and thin, fragmented elastic fibers. Verhoeff staining, ob. 60x

Our observations support the quantitative data obtained on CT images. In the masseteric region the superficial fibroadipose layer has an average thickness of 4.32 +/- 2.9 mm, and the deep fat layer is very thin, 0.33 +/- 0.48 mm. SMAS appears as a hyperdense line intimate to the gland, with a thickness of 0.76 +/- 0.43 mm (Figures 2.6., 2.7. and 2.8.).

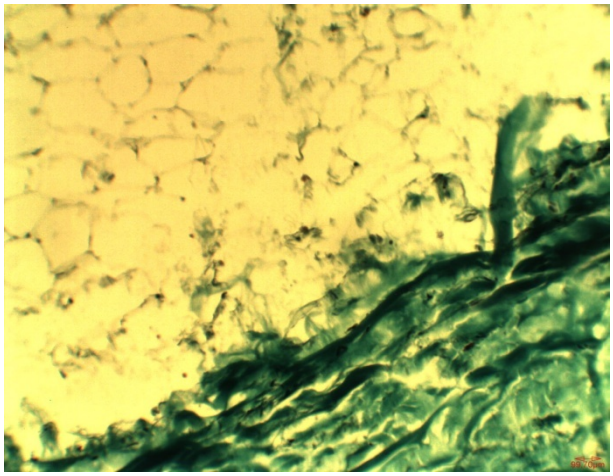

Figure 2.8. SupraSMAS fibro-adipose tissue, well-represented, in the parotid region. Szekely staining, ob. 20x

In parotid region, superficial fascia presents numerous fat lobules and is part of the SMAS of head and neck, together with the mimic muscles, blood vessels and nerves that run across (Figure 2.9.). They are separated by vertical supraSMAS and horizontal subSMAS fibers. The presence of infraSMAS horizontal tract has allowed us to obtain a layer of cleavage and surgical approach between the superficial and deep fascia. Highlighting of vertical tracts allowed us to affirm that it is a common feature with neighboring facial regions. These vertical tracts permit the removal and reattachment of a SMAS flap and,

obviously represents the anatomical substrate of a major cosmetic result in parotidectomy techniques based on the existence of SMAS.

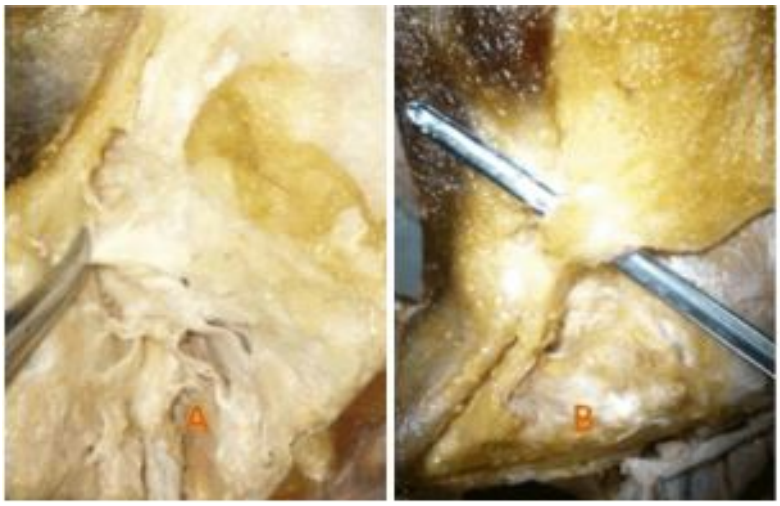

Figure 2.9. Parotideo-masseteric fascia retaining ligament: the superior region (A) and the inferior region (B). Dissection specimen.

In the posterior part of the parotid gland, the superficial fascia exhibits a condensation that forms a true hill for the gland. Here, the facial nerve, the facial artery and the external jugular vein enter the parotid gland. After dissecting the superficial and deep parotid fascia, we identified the branches of the facial nerve. They follow the fibrous expansions of the superficial fascia.

Thus, we can assert that the superficial fascia forms tunnels for the terminal branches of the facial nerve, which allows us to map and preserve them. Continuing in-depth dissection, we identified the formation of the external jugular vein and the terminal segment of the external carotid artery. Vascularization and innervation of face skin is done directly through the superficial fascia. Essential constituent of the SMAS is superficial fascia (Figure 2.10.), which continues in all regions of the face, only muscles being different.

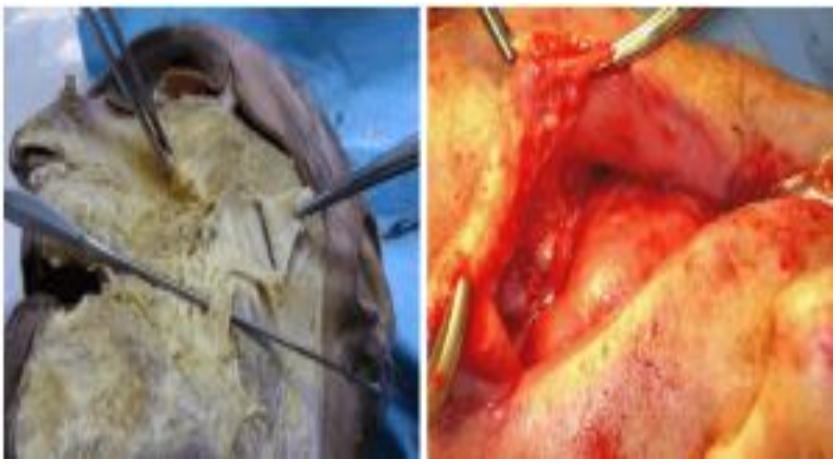

Figure 2.10. Removing the superficial muscular aponeurotic system and parotid fascia: dissection specimen (left) vs. intraoperative image (right).

Blood vessels and terminal branches of the facial nerve, crossing each several regions of the face are closely related to the superficial fascia, which mechanically protects them.

The classical theory regarding origin of parotid fascia as derived from deep facial fascia is invalidated by its anterior continuity with platysma muscle fascia. In upper of the cheek, it makes the transition to the zygomatic region. Morphological, macroscopic and mesoscopic differences between the two regions stand out as a net line, "as a border strip" (Figure 2.11.).

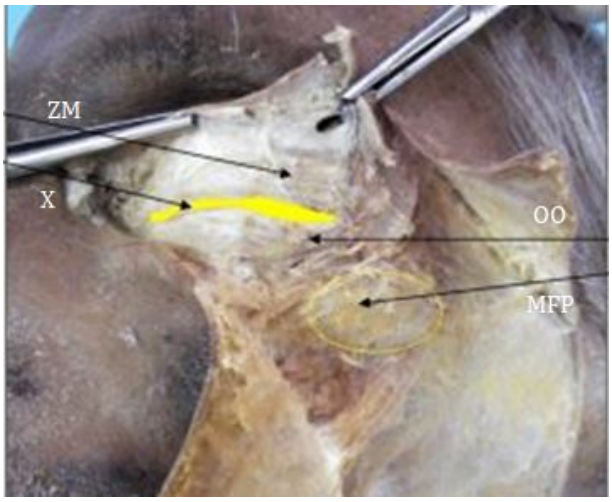

Figure 2.11. The boundary between the upper and lower face (X) given by the great zygomatic muscle; stratigraphy of infraorbital region – great zygomatic muscle (ZM), inferior fascicle of orbicularis occuli (OO) muscle; molar fat pad (MFP).

Although SMAS is intimately applied to the surface of the parotid gland, a thin, well-defined parotid fascia is identified between the gland and the SMAS. Superficial fascia facilitates adherence of mimic muscles to the dermis and deep fascia (Figures 2.12. and 2.13.).

This suggests that oral superficial fascia will follow chewing movements of the masseter muscle. SMAS is well represented, with thick collagen fibers condensations, mostly disposed longitudinally, with dimensionally reduced interstitial spaces (Figures 2.14.), in continuity with the neighboring regional fascias.

The blood vessels are found only on the periphery of the lamina and are very small. Distance to the deep, parotideo-masseteric fascia, is reduced, the fat infraSMAS layer being also reduced and represented by a very thin lamina of adipose cells, crossed by thin collagen fibers obliquely oriented. Elastic fibers are completely absent.

Fibro-adipose superficial layer is well represented with connective fascicles, observing numerous fat lobules separated by connective tracts and almost vertical or slightly oblique in one direction or another. There are predominantly medium-sized collagen fibers and rare elastic fibers.

Figure 2.12. SMAS adhesions to deep fascia that secures the side of the parotid region. Dissection specimen.

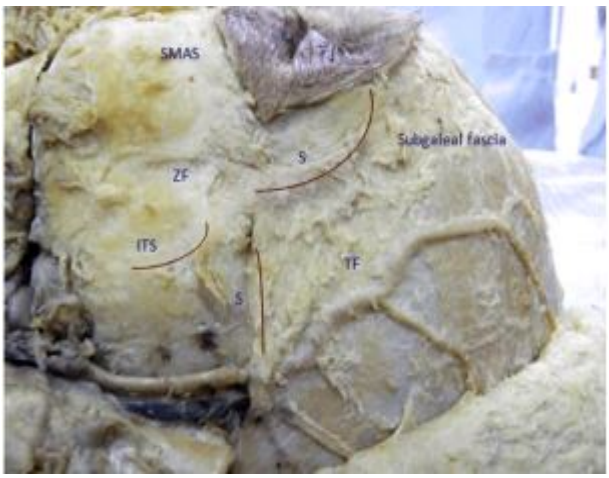

Figure 2.13. Ligamentary supraorbital (S) adhesion, superior temporal septa (STS) and the inferior one (ITS). SMAS: Superficial muscular aponeurotic system; TF: Temporal fascia; ZF: Zygomatic fascia.

Our observations support the quantitative data obtained on CT images from the group of patients that underwent surgery.

In parotid region, fibroadipose superficial layer has an average thickness by the 4.31±3 mm, and deep fat layer is very thin, 0.34±0.47 mm. SMAS appears as a hyperdense line in the intimate relation to gland, with a thickness of 0.44±0.74 mm.

The superficial layers of this region have different thicknesses: the supraSMAS fibrous tissue is well represented (Figure 2.15), the SMAS is a structured, dense organized connective tissue, the infra SMAS fat tissue forms a very thin lamina under which lies deep parotid fascia.

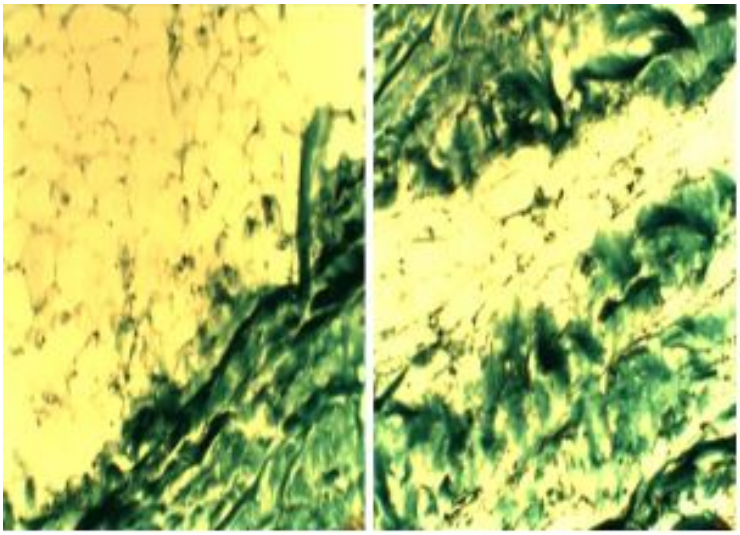

Figure 2.14. Fibrous-adipose supraSMAS (left image) and infraSMAS (right image) tissue is well represented in the parotid region. Szekely staining, ×200.

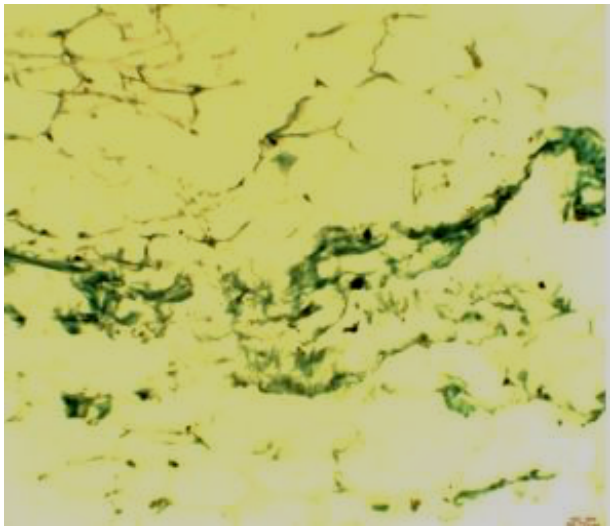

Figure 2.15. SMAS fibrous layer, well represented in the parotid region.Szekely staining, ×200. SMAS: Superficial muscular aponeurotic system.

CT investigation shows that the boundary between the two lobes of the gland is considered to be an area of continuity between the superficial and deep fascia (which forms the posterior capsule). Posterolaterally, parotid fascia is continued by a conjunctive part, which interconnects with superficial fascia and sternocleidomastoideus muscle fascia. Anteriomedially, fibrous tissue forms tunnels through which branches of the facial nerve are running (Figure 2.16.).

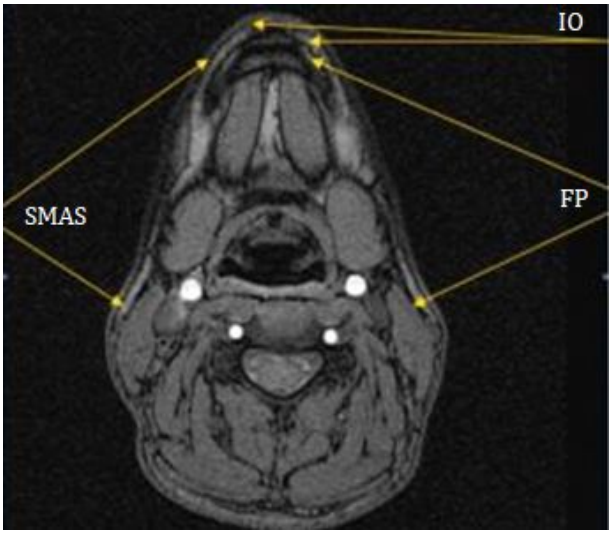

Figure 2.16. Horizontal section through the lower edge of the mandible; SMAS and deep fascia (FP) between the parotid and mental region; transSMAS insert of the orbicular mouth, inferior fascicle (IO).

2.2.3. Discussions

Parotid masseteric SMAS is well personalized and may contain some muscular fibers and nerves fibrous tunnels (Gola, 2005; Gola, 1994). It covers parotid gland and masseter muscle (Meningaud, 2006; Stathopoulos, 2018; Delmar, 1994; Gosain, 1993). Riolan in Le Double signaled „portio musculi cutanei supra parotidem ad aurem ascendentis" (Double, 1897), a part of the platysma muscles that ascends to the ear, passing over the parotid. It is fixed to the auricular cartilage and establishes deep adhesions with the parotid capsule. In the area located inferior to manibular angle and in mastoid region SMAS adheres to the superficial cervical aponeurosis that covers the sternocleidomastoideus muscle.

In the masseteric region, the superficial fascia features numerous lobules. They are superimposed by vertical fibrous tracts and infra-SMAS these tracts have a horizontal disposition. In the posterior part of the parotid gland, the superficial fascia exhibits a condensation that forms a true hill for the gland. At this level, the facial nerve, the facial artery and the external jugular vein enter the parotid gland.

Jugal SMAS is thin, discontinuous and difficult to be dissected; it becomes gradually thinner from the posterior to the nasolabial above and does not go beyond the groove. It contains the risorius muscle that moves paracomisually and pulls it back in the smile. This muscle develops in SMAS thickness, before masseter aponeurosis, but without inserting it.

SMAS forms together with skin a functional, tegumentary, adipose and neurovascular unit, physiologically inseparable, cervicofacial cutaneous muscular aponeurotic unit (UCMA) (Taylor, 1987).

On axial CT, SMAS appears as a relative hypertension, tortuous line between superficial fibrous tissue and deep adipose tissue that are hypodense. Quantitative measurements performed on CT by researchers (Pearl, 1983; Whetzel, 2015) showed regional differences in thickness of the superficial layers of the face.

Quantitative measurements were made on the representative microscopic sections and were taken from the microscope using an image acquisition system (a video camera connected to a PC), after which the PRODIT 5.2 professional program was applied. This interactive digital program has enabled many measurements to be made by choosing the desired quantitative method from the menu, automatically calculating the results.

In the parotid region the superficial fibroadipose layer has an average thickness of 4.32 +/- 2.9 mm and the deep fat layer is very thin, 0.33 +/- 0.48 mm. SMAS appears as a hyperdense line intimate to the gland, with a thickness of 0.76 +/- 0.43 mm. At the cheek level, the superficial fibroadipose layer is very well represented (5.57 +/- 1.17 mm), the thicker layer is thinner, 2.94 +/- 0.62 mm, and SMAS recognizable slightly (2 , 94 +/- 0.62 mm). At the level of the nasolabial groove the superficial fibroadipose layer is poorly represented (0.37 +/- 0.06 mm), the deep fat layer has an average thickness of 2.15 +/- 0.63 mm, while SMAS continues with muscles of facial expression, also with average thickness (2.41 +/- 0.05 mm).

On MRI images, SMAS appears as a continuous hypotensive line in T1 and T2 weights, from the parietal region to the nasolabial sulcus. It includes the muscles of facial expressions in the cheek regions and in the nasolabial sulcus.

Our anatomo-imaging study confirms the architectural composition of the face from multiple layers of tissue that connects the facial muscles to the dermis. Aspects encountered on MRI images at various incidences support microanatomical observations, obtained on sections at various levels, according to which, the arrangement of SMAS suggests a gradual centrifugal thinning to the adjacent regions.

In the parotid region, and the deep fascial plan it is formed by the parotid-vesicular fascia.

Also here are important force vectors represented by masticatory movements which are highly affected in lesions of the facial nerve when reconstruction technique are required.

In the parotid region, the most important neighboring relation of the parotid fascia is that with the branches of the facial nerve and the maxilla. These nerves fibers are accompanied by branches of the external carotid artery. Preserving them is the greatest care of a surgeon who intervenes at this level (Siemionow, 2006).

Nowadays, by preserving these neurovascular bundles, it is possible to operate a complete face transplant. Data obtained by imagistic records are consistent with those in the literature – quantitative measurements performed on CT (Macchi, 2007) showed regional differences in thickness of the superficial layers of the face. At the parotid level, the SMAS has individual features and also common morphofunctional characteristics with other facial regions (Zigiotti, 1991). The common features are that superficial fascia facilitates the adhesion of the mimic muscles from the region to the dermis, forms portvas and portnerve blades and presents an adipose supra and infraSMAS layer.

Particular aspects of the parotid region, which we highlighted in this study, refer to the fact that the superficial layers have different thicknesses, the supra SMAS fibrous tissue is well represented, the SMAS is an ordered, dense organized connective tissue, infraSMAS adipose tissue forms a very thin blade under which there is a deep parotid fascia. The latter has a much dense structure, the collagen fibers constituting a dense structure with

homogeneous masses in which separated fibers are not individualized. We can affirm they have a lamellar disposal (Pearl, 1983; Jost, 1984).

This reveals that the oral superficial fascia will follow the masticatory movements of the buccinator and masseter muscles, but also those of the great and small zygomatic muscles. These muscles are the infraSMAS layer in parotid region and thus take part in the formation of a unitary complex together with the superficial fascia. The parotid SMAS continues with that of the adjacent regions, being fixed superiorly by the lower part of periorbital septum and zygomatic arch (Wassef, 1987).

Medially, it adheres to the maxilla through the fibrous condensation of the nasolabial ditch. Inferiorly it is inserted at the lower edge of the mandible, from where it continues with the surface of the platysma.

Laterally, it forms the anterior sheet of the parotid fascia. The great zygomatic muscle adheres to its front face at the superficial fascia. The latter has a much dense structure, the collagen fibers constituting a dense structure with homogeneous masses in which fibers (Macchi, 2010) are not individualized.

We can say, they have a lamellar disposal and elastic fibers are thin and fragmented. Therefore, elevation of the SMAS is concomitant with the muscle (Mendelson, 1995). Although it is intimately applied to superficial surface of the parotid gland, a weak but distinct parotid fascia is identified between the gland and the SMAS. The CT examination of patients supports the previous results, showing that, reaching the parotid gland, the superficial fascia gives rise to the anterior capsule of the gland, data that corresponds to literature (Baek, 2007).

The existence of a SMAS layer on the parotid capsule allows the application of techniques that have been used in facial lifting procedures and parotid tumors. This is a novelty in the techniques of maxillofacial surgery and improves the results of interventions at this level. The identification of the facial nerve in its quadrilateral, its intraparotid dissection, and especially the fascial tunneling of its terminal branches, allows a better preservation of the nerve, in the main patient benefit (Ikoma, 2014).

The decrease in the incidence of postoperative infections to less than 1% and the higher aesthetic results (Wong, 2017) recommend the use of the concept of the single surface layer demonstrated by us in parotid surgery.

Musculofascial superficial aponeurotic portion of the system is distinguished in the subcutaneous fat layer, only the posterolateral areas of the face where there is a deep fascial support, well-tensioned functional.Collagen fibers have ordered disposition on successive longitudinal and transverse plans, or they perceive their individual structure, forming layers of varied shapes and sizes, most obviously at the level of the modiollus.Muscle fibers belong to the superficial layer of the skin that form SMAS in some areas, leave it or cross to the deep surface or the osteoperostic plan. Masseterin SMAS is a dense lamina, particularly with collagen fibers, with rare muscle fibers. SMAS layer has a mixed structure, with very small quantitative differences between connective tissue and muscles. The masseter fascia is a surgical entity because it is an important in facial reconstructions.

In the posterior part of the face, facial nerve branches have a profound situation, finding themselves into a musculo-fascial layer between the superficial and deep fascia (parotideo-masseteric) or intraglandular. Musculofascial superficial system is an

independent plan that is individualized subcutaneous as a fat layer, just under the posterolateral areas of the face (parotideo-masseteric, oral and frontal), where there is a deep fascial support very well tensed functional.

2.2.4. Conclusions

Although SMAS is intimately applied to the superficial surface of the parotid, a distinct but distinct parotid fastia fascia is identified between the gland and SMAS. At the jugal region of SMAS, it protects the facial vasculo-nervous system by forming a fibrous lining around it. SMAS forms the anterior capsule of the parotid gland, from where the posterolateral side continues with the sternocleidomastoid muscle fascia, and antero-medial with fibrous tunnels for the branches of the facial nerve. Collagen fibers have ordered disposition on successive longitudinal and transverse planes, or they perceive their individual structure, forming blades of varied shapes and sizes, most obviously at the level of the modiol.Muscle fibers belong to the skin of the superficial layer that make up SMAS in some areas or leave it or cross to the deep face or the osteoperostic plane. Parotid SMAS is a dense lamina, fiber, particularly collagen, with rare muscle fibers, and SMAS to the upper lip has a mixed structure, with very small quantitative differences between connective tissue and muscle.

2.3. Anatomic approach on buccal fascia

Superficial fascia of the different regions of the body is universally accepted that it exists and proceeds from one region to another, except the face. In parotid, masseteric and jugal regions its existence is evident, while in the middle regions of the face - nasal and oral - it is long disputed.

Not long ago (Tessier, 1989) it was proposed the existence of a morphofunctional cervicofacial system formed by platysma muscles, mimic muscles and superficial cervical and facial fasciae. At the facial and anterior cervical regions, the superficial fascia does not have a role of separation between superficial muscles and skin, but a role of cohesion and connection between them.

In our previous studies we have investigated the disposition of this musculofascial structure in the lateral regions of the face, dissecting and exploring the fixation means for the soft facial structures to the proximal periosteum. We have identified the ligamentary adhesions, ligaments and septa that fix SMAS to the bone, these being the main mechanism of antigravitational support for the soft facial tissues. From this point of view, the existence and particularities of the superficial musculoaponeurotic system plays a crucial role against the phenomenon of „aging face".

We have also identified and researched the morphology and function of SMAS in the Moebius Syndrome. Congenital atresia of the facial nerve leads to important functional deficiencies of the perioral muscles which, over time, affect adjacent regions by elongation of fixation means and appearance of superficial soft tissue prolapse. Surgical reconstruction

technique which use the temporal muscle tendon transposition is based on the existence of an oral superficial musculoaponeurotic system.

In the following lines we will approach the oral structures in anatomo-functional terms, from a lesser-known perspective: the existence of a unique superficial layer that is closely related to both the skin and subjacent muscular layer.

In support of this theory there are several aspects, such as:

- It explains the functional mechanisms of the facial expression muscles;
- Development of the superficial face and neck muscles and fasciae shows that the development of the facial expression muscles, superficial and profound adipose tissue, facial nerve and parotid gland is the result of some divergent movements and migratory confluences (Standring, 2016), which eventually led to anatomical shaping of a superficial and unique layer at face-level;
- It explains the anatomo-functional mechanisms of the „aging face" phenomenon;
- Nowadays cosmetic surgery would not be possible without SMAS. Old cosmetic surgery techniques, which did not take SMAS into account (or maybe it was not known at that time), could cause downright mutilating scars to patients.

2.3.1. Material and Methods

Our study was carried out on a material consisting of twenty-four dissected hemifacieses formalized and dissected at the Institute of Anatomy „Ion Iancu" within University of Medicine and Pharmacy „Grigore T. Popa" Iasi. The macroscopic study on the dissection specimenswas performed using the SOM 62 Kaps operator microscope owned by „Ion Iancu" Institute of Anatomy, University of Medicine and Pharmacy „Grigore T. Popa" Iasi. There were identified the following overlapping layers on the dissection specimens: dermo-epidermic, subcutaneous adipose, superficial fascia, superficial muscular layer, deep fascia, profound musculo-glandular elements and periosteum layer, partially sampled in order to stabilize the fragments. The conclusive aspects taken over by an image acquisition system were subsequently examined and processed to spot regional topographic differences.

As regards the qualitative micro-anatomical study of SMAS within the topographic pattern of the face, we sampled soft tissues from oral region, starting from the skin to the bone layout, in the form of small blocks. The sectioning was made perpendicular to the epidermis surface in order to be able to examine the correct sequence of the planes. Sampled fragments were processed using paraffin and H&E stained technique as well as special techniques for connective and muscle tissue (Verhoeff). For stereology technique we used PRODIT 5.2 programmein order to make quantitative measurement of connective and musculartissue.

Our casuistry also included a group of fifteen patients imagistically explored for aesthetic surgery purposes or for congenital malformations surgical treatment (Moebius syndrome, congenital facial nerve paralysis) in the Maxillofacial Surgery Clinic within "St. Spiridon" Emergency Clinical Hospital, Iasi – a University based hospital.

MRI is the imaging exploration method that provides the most conclusive images on the cervicofacial soft structures. It is able to demonstrate the stratygraphic anatomy data in detail, obtaining images similar to CT but with a better differentiation of the soft tissues.

2.3.2. Results

Oral region has as its main feature the existence of an infraSMAS space filled with adipose tissue. This space is a way of spreading for an infection into neighbouring regions. The infraSMAS connective tissue from this level contains fibres organized as conjunctive tracts which separate the fat lobules (Figure 2.17.).

Its structure includes also muscle fibres from the buccinator muscle. Muscle adhesion to the skin through SMAS leads to the conclusion that SMAS actively intervenes in the masticatory act, pulling the skin in direction of the movements of masticatory muscles (Figure 2.18.).

Starting from the zygomatic arch and progressing to the mandible, SMAS and infraSMAS tissue are giving the cheek appearance by the amount and shape of the adipose tissue, which they delimit it. Here, it also offers skin firmness at the level of the deep planes.

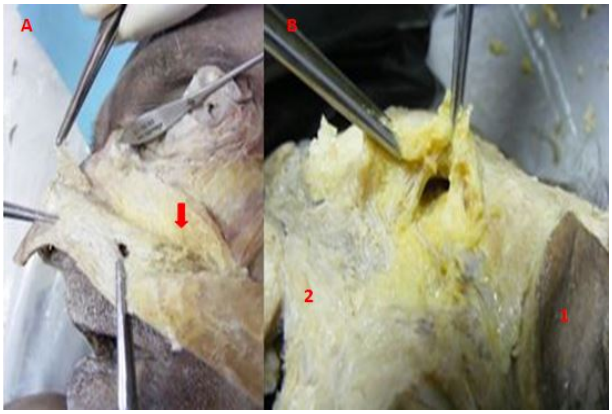

Figure 2.17. Connective-adipose tissue from the cheek, prebuccinator and perioral region. Red arrow from picture A points the examined region from picture B. 1 represents the infraorbital region and 2 the labial commissure. Dissection specimen (SOM 62 KAPS microscope, x20 oculars), 10/1 scale in the right image.

Oral muscles adhere intimately to the deep surface of the skin, crossing the superficial fascia. At this level, into the thickness of the musculoaponeurotic system there is adipose tissue trabeculated by conjunctive septa and labial vascular nervous structures. The insertion is firm, almost impossible to dissect. Because of this, superficial fascia is extremely difficult to be shown by classic anatomical dissection (macroscopic). Thus, it is preferred to choose the operator microscope for examination of this region.

We found the continuation of the SMAS with the superficial fascicles of orbicularis oris muscle, suggesting that this layer represents SMAS into superior and inferior lips, separated by the overlying fascial layer (Figure 2.19.).

We have to emphasize once again the importance of zygomatic ligament, which also contributes to raising the angle of the mouth, applying traction to the superficial fascia of the

region. Traumatic injury or its involvement in another pathology may lead to the appearance of some deformities when attempting a facial expression. Its structure is unitary, robust, with connective fibers in its axis (oriented in the superior-medial direction, in the sense of the traction forces vectors acting on it), macroscopically visible. It has two portions: medial (periorbital) and lateral (tragally) (Figure 2.20.).

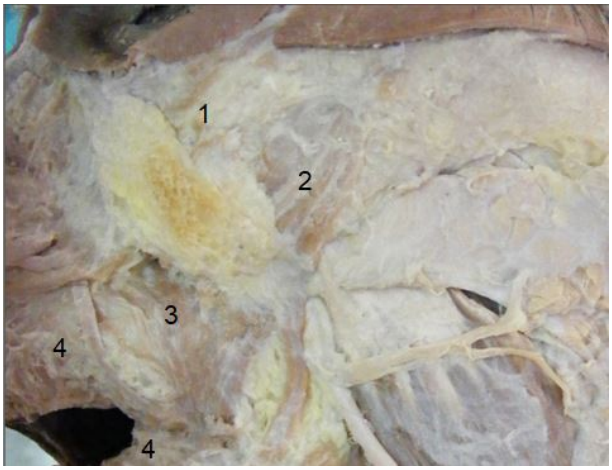

Figure 2.18. Muscles of facial expressions at the mouth angle: 1 - zygomaticus minor, 2 - zygomaticus major, 3 - levator anguli oris, 4 orbicularis oris. Dissection specimen (SOM 62 KAPS microscope, x20 oculars), 3/1 scale.

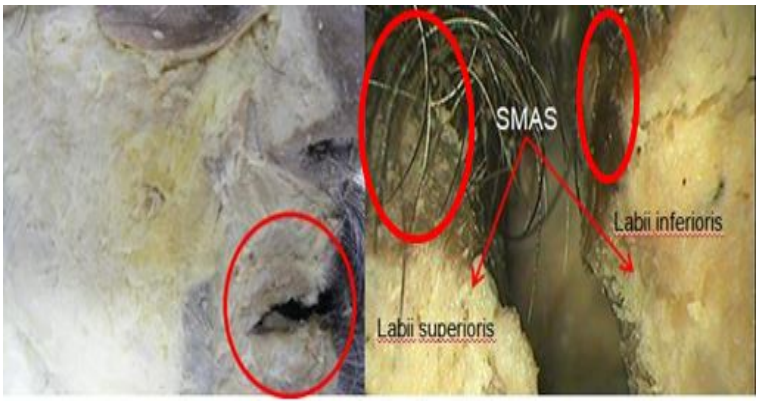

Figure 2.19 SMAS at the level of the lips. Red circle in the left picture points the interest area, magnified into the right one. The two red circles from the right picture mark the areas of both lips where SMAS ends. Dissection specimen (SOM 62 KAPS microscope, x20 oculars), 20/1 scale.

Dissecting downward to inferior lip, we have easily revealed a musculo-conjunctival infradermic layer on anterior mandibular surface, which offers attachment for mental muscles.

Usual staining, specific to connective and muscular tissue, reveals that collagen fibers at this level are thinner, rarefied and placed in disorder, along with more elastic fibers and with muscular fibers longitudinally disposed in the SMAS structure at the upper lip level (Figure 2.21.).

Interconnecting muscular fascicles of orbicularis oris muscle, we will find collagen fibers with the same longitudinal disposal, from which thin paths descend to the skin (Figure

2.22.). The conjunctive fibers of SMAS, medial of the nasolabial groove, have longitudinal disposal.

At the angle of the mouth, the numerous collagen fibers lose their fibrous structure making dense clamping strips, which also provide resistance to muscle contraction (Figure 2.23.). They are intertwined with fascicles of muscular fibers, while the elastic fibers are almost absent.

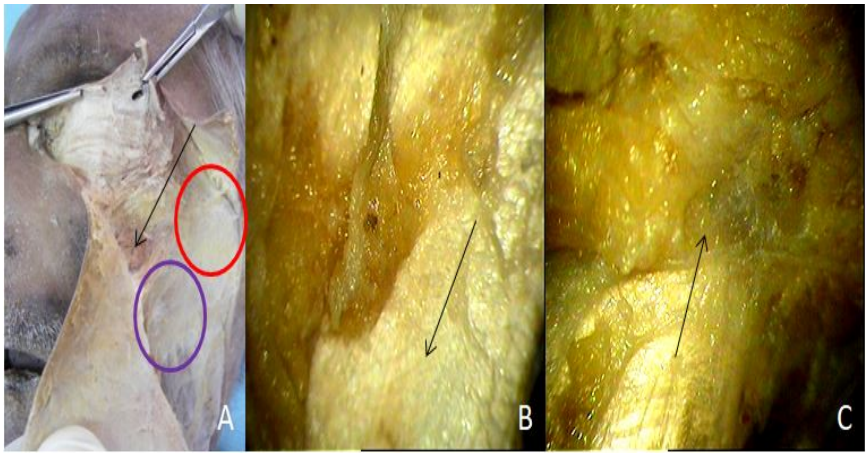

Figure 2.20 Image A shows within the two circles both parts of zygomatic ligament. First part, marked by the red circle represents the tragal part, magnified in picture B. Purple circle represents the medial part which adheres on the skin of the cheek, magnified in picture C. The arrow points the direction of ligamentary fibers, from zygomatic arch to the cheek, in all 3 pictures. Dissection specimen (SOM 62 KAPS microscope, x20 oculars), 20/1 scale in B and C images.

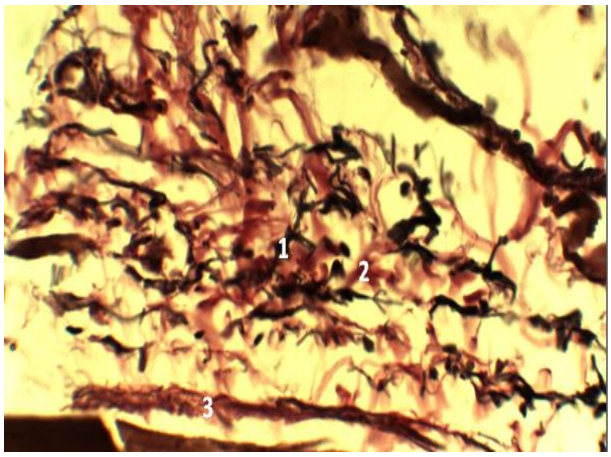

Figure 2.21. Thinner collagen fibers (1) and more elastic fibers (2) along with longitudinal muscle fibers (3) in the SMAS structure from the upper lip, middle third level.Col. Verhoeff, ob. 40x.

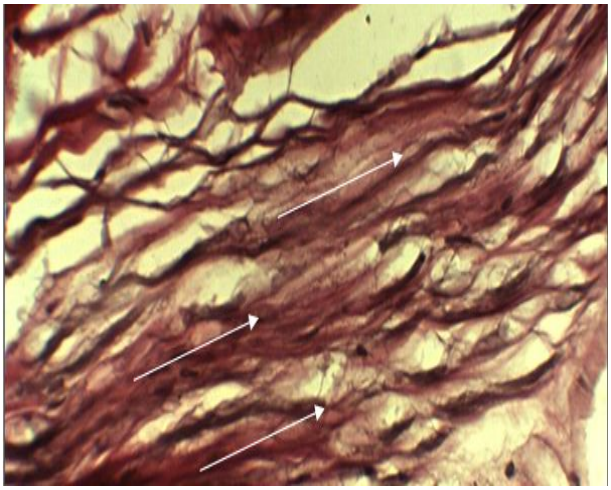

Figure 2.22. SMAS medially to nasolabial sulcus with longitudinal disposal of collagen fibers, marked by white arrows. Col. Verhoeff, ob. 40x.

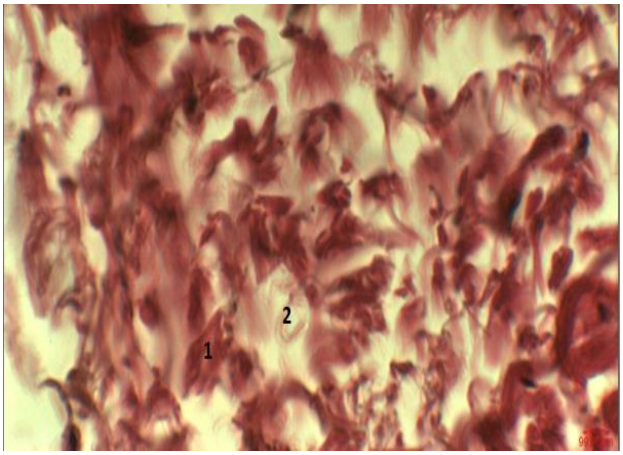

Figure 2.23. The SMAS structure at the mouth angle, with many interwoven collagen fibers (1) and with elastic fibers almost absent (2). Col. Verhoeff, ob. 60x.

On the lamella stained with Verhoeff`s method we quantified the volume percentages of connective tissue, muscular fibers and interstitium compared with the other components, including adipose tissue using.

Quantitative measurements show the following volume percentages quantified by stereological examination of the lamellas:

- connective tissue – 45.56%;
- muscular fibers – 38.15%;
- interstitium – 16.30%.

SMAS cannot be identified in the free margin of lips, both macroscopically and microscopically. Verhoeff staining shows the lack of elastic fibers and the fact that the interstitial tissue prevails.

The MRI images show that above the superior margin of the mandible, the superficial fascia (SMAS) also behaves differently:

a. medially, it gives insertion to orbicularis oris muscle (inferior fascicle) and then to depressor anguli oris muscle (Figure 2.24.);
b. laterally, it becomes mobile, ascending first above jugal fat pad, buccinator and then masseter muscle (Figure 2.25.).

At superior lip, superficial layers become fixed once again. This happens due to insertion of orbicularis oris muscle (superior fascicle) and levator labii superioris on the profound surface of the skin, transfascially.

Even if superficial fascia gets thinner and thinner to modiolus, its thickness is still enough to appear as a clear tissue strip on MRI. The same thing is revealed on dissected specimens.

Ascending to the nasal septum base, the two fasciae (superficial and profound) are united into a dense conjunctive structure. The more we are going to the nasolabial groove in its medial part, the more clearly superficial fascia becomes.

It provides protection for the superior branch of angular artery and for superior labial fascicles of buccal branch from facial nerve. Injures of these branches of the facial nerve or of its trunk cause static deformities of this region, alimentation and phonetic disorders, depending on the scale of the injury.

These disorders are reflected in the skin insertion of the muscles and the continuity of SMAS towards the other regions that involves their step by step transmission (Figure 2.26.).

2.3.3. Discussions

SMAS relationships with nasolabial groove are still controversial. Literature studies (Mitz et al., 1976) described anterior continuation of SMAS into the superior lip, overlying the muscular layer. Barton et al., (1992) described a thin fascial layer covering the zygomatic muscles and extending into the superior lip, but they do not identify the subcutaneous extension of SMAS. Pensler et al., (1985) distinguished a SMAS layer which is medial to the nasolabial groove, whereas Yousif et al., (1994) consider that there is an adipose supraSMAS layer on the cheek and superior lip.

On the dissected specimens we have identified, starting from superficial to profound, the following layers:

- *cutaneous, with a denser profound part, acting for insertion of the facial expression muscles;*
- *subcutaneous adipose layer;*
- *superficial facial muscular layer, arranged as follows:*
  - ***oral region**: zygomatic, rhizorius, orbicularis oris, levator labii superioris, and at the angle of the mouth, modiolus, levator anguli oris and the buccinator;*
  - ***mental regi**on: depressor labii inferioris, depressor anguli oris, platysma, mentalis.*

This particular SMAS architecture into oral region is the most important mechanism of support against the “aging face” phenomenon, preventing the occurrence of perioral creases, and downturned of oral commissures (Jeffrey et al., 2015).

Anatomy studies conducted with focus on superficial muscular and fascial structures support the Duchenne`s statement in 1862: "*the law governing the expression of the human face can be discovered by studying the action of the muscles*" (Duchenne de Boulogne GB, 1990).

Surgical techniques of facial rejuvenation and dynamic resuscitation of the oral sphincter are based on the existence of this functional musculoaponeurotic facial system (Pidgeon et al., 2017

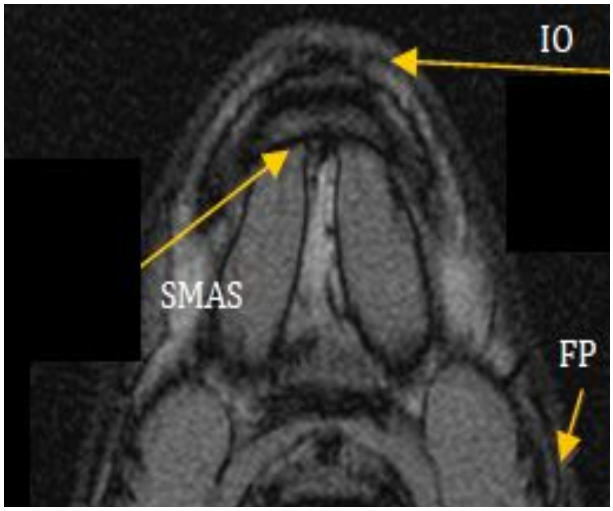

Fig. 2.24. MRI horizontal section through inferior mandibular margin; SMAS and profound fascia (PF) between parotidian and mental region; TransSMAS insertion of orbicularis oris, inferior fascicle (IO).

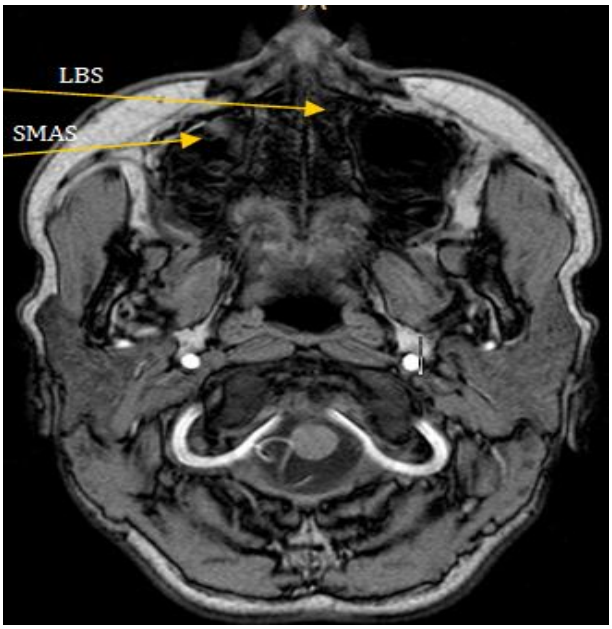

Figure 2.25. Transversal MRI which illustrates TransSMAS insertion of levator labii superioris muscle (LBS).

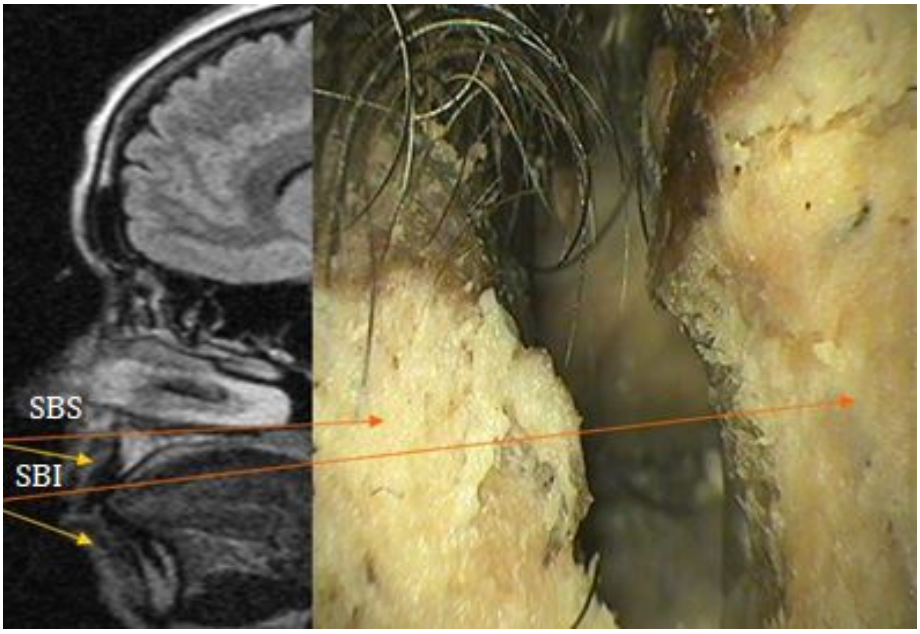

Fig. 2.26.SMAS at superior lip (SBS) and inferior lip (SBI).

In this context, SMAS, the musculofascial sheath that goes downward the frontal muscle to platysma, plays an essential role, acting as a "**facial muscular contraction amplifier**". In fact, it is a distributor of muscle contraction towards the skin: each muscle contraction follows a preferential direction in the network.

An infinite number of actions are possible because, on the one hand, SMAS conveys the contraction of the muscles along the parallel network towards the skin area, and on the other hand it transmits the resulting effect into a perpendicular direction towards the skin, through fibrous expansions from SMAS towards skin. Lifting of the upper lip for facial rejuvenation is based on these features of the regional muscles (Bai-lin Pan, 2017).

SMAS is present in the upper lip and represents the superficial portion of the orbicularis oris muscle but it is not revealed at the free margin of the lips. The main feature of this region is the fixity of the superficial structures to the profound ones. This feature is the basis for the principles of oral facial lifting (Le Louarn et al., 2006; Fogli, 2017).

This is caused by strong and flared insertions of the muscles from this level to the profound face of the skin. We can state that it is the real visceral segment of the face. Some oral muscles (orbicularis oris fascicles) play the role of sphincter of the orifice they surround while the others are functional extensions of them.

The more we move toward the midsagittal plane, the thinner the skin becomes. The same disposition is revealed in the subcutaneous adipose tissue, with the mention that the supraSMAS adipose layer is almost non-existent. The superficial and deep fasciae lose their elasticity, being composed of dense connective tissue.

2.3.4. Conclusions

Facial SMAS is a unitary structure that fixes the dermis to the facial bones providing a multiligamentary fibrous support system. We showed that oral SMAS exists and facilitates

the skin insertion of the oral muscular apparatus. Through SMAS, the oral muscles can exercise their controlled contraction and sphincter function. Zygomatic ligament is the key structure in the dissection process for successful mobilization of the middle part of the face. The facial nerve branches are located between the muscular layer and SMAS. At the upper lip, SMAS has a mixed structure with quite small quantitative differences between the connective and muscular tis- sue. SMAS is also realizing connective tunnels for the branches of the angular and buccal artery. These anatomical findings could be useful for the understanding of the SMAS concept and when per- forming various types of facial surgery.

2.4. Further enhancements

Our study on superficial cervicofacial musculoaponeurotic system have demonstrated the existence of a a complex structurally and functionally formation at the face and anterior neck.

We have divided the face into three distinct regions according on SMAS disposal: median, medials and laterals. Each of these regions presents morpho-functional peculiarities of SMAS that reverberate directly into clinical implications.

In each of these regions the peculiarities refer both to the means of fixation of the SMAS in the proximity and to its architectural composition (type, orientation and quantity of fibers in its structure).

In the median region of the face, the SMAS organization has particularities related to its distribution and orientation around the orbits, nasal fossils and oral cavity.

Periorbital and nasal adhesions of SMAS have direct implications in plastic and reconstructive surgery. SMAS insertions in the nasolabial ditch and perimeter of the oral sphincter also play an important functional role, actively intervening in speech and nutrition.

Intermediate regions represents the link between the middle and the lateral ones on the same side. In its depth the adipose tissue is trabecularly organized in the zigomatic and jugal regions, actively intervening in the antigravity support of superficial soft structures.

On the sides, SMAS is firmly attached to the periosteum, providing anti-gravity mechanical support and facial dynamics.

It is important to continue this research through both evolutionary and functionally studies of muscle and fascial structures involved in SMAS formation. Evolutionary studies refer to retrospective and prospective research of patients diagnosed with congenital facial paralysis.

The results of this study are perfectible by using advanced imaging techniques such as fMRI, structural ultrasound and elastography on facial expressions muscles, platysma muscle and cervicofacial superficial fascia.

3. ADVANCES IN SINGING VOICE

Brief history of singing voice

The opera voice has always attracted the interest of the researchers both from the artistic and medical field, even though the latter may be more preponderant. The complexity of the physiological processes and the functional adaptation of the morphology of the anatomical neural structures involved in this process are not fully known so far.

The study of vocal training mechanisms is not deepened in medical faculties or in related medical specialties because their main concern is the pathology of phonetic organs - phoniatrics.

All of these findings have proved the scientists's need to systematize these data and have made us to pursue the research directions in this vast field using modern techniques and devices.

Until 1950, it was thought that the vocal muscles are formed by a set of fibers in parallel to the homolateral vocal ligament. In the same year (Goerttler, 1951) it has been shown that this muscular organization does not exist. In fact, these muscles are inserted on the vocal ligament "in teeth of comb". These muscles form two cross systems that insert on the elastic cone around the vocal ligament and are named after the german professor who described them first - Goerttlerian fibers.

The basic explanation of sound formation is given by myoelastic Van den Berg theory (Bernoulli effect), which states that the vibration of the vocal folds is given by the relaxation of the vocal muscles and by its tonus to the decrease of the infraglottic pressure with the adjoining vocal folds (Van den Berg, 1958).

Husson's neurochronaxic theory demonstrates that vibration of vocal folds is given by the periodic active stimulation of the recurrent nerve, impulse after impulse (Husson, 1951). This theory basically applies to each glottic muscle served by the recurrent nerve, an individual nervous fascicle from the common recurrent trunk. It explains the homorhythmic vibrations of the vocal folds with that of the recurrent impulses that occur even in the absence of a glottic air column (Husson, 1960). The same has been previously demonstrated by other researchers (Heymann, 1933; Portmann, 1955; Laget, 1953; Piquet, 1956).

Formation of laryngeal sound (the fundamental frequency of the voice) takes place through a sequence of ultra-short, rhythmic and rapid contractions of the Goerttlerian fibers of the vocal folds, each contraction moving the two vocal cords and producing a ”vibratory” phase of the glottal opening (Fabre, 1957).

Regarding the palatoglossal muscle, there is a dispute between anatomists about his belonging to the extrinsic tongue muscle group that dates from the beginning of the 20th century. Thus, after an animal experimental study, Egyptian physiologist and histologist Shafik Abd-El-Malek does not include this muscle among the extrinsic muscles of the tongue. He explains his conclusions by the fact that "the movements of the tongue appear as a result of the partial or total contraction of different muscles, which can act together or antagonistically. Due to the complicated interconnection of these muscles, especially the

intrinsic ones, it has been difficult so far to determine the individual action ” (Abd-EL-Malek, 1938).

Classical anatomy describes palatopharyngeal muscle as being innervated by the IXthcranial nerve (glossopharyngeal). The palatopharyngeal muscular fascicles have an inferior lateral direction and to posterior, from their origin to the palatine veil insertion. They pass deeply to the superior constrictor of the pharynx and form a mucosal fold at the level of the nasopharyngeal isthmus called Passavant's crest.

Doctor Gustav Philip Passavant (Passavant, 1932) was the first that described this anatomical concept and the material written by him was first printed after his death in 1932 in Biographisches Lexikon. The same researcher also described for the first time the feeling of sore throat during phonation as given by the functional dissociation of the horizontal fascicles from the palatinal muscles.

Up to nowadays, it has not been possible to accurately state from a scientific point of view whether a person is born with certain anatomical variations that predispose him or her to be able to perform in singing, or these skills are acquired. The phonation in canto voice is different from the usual one or even from the theater phonation. These differences are found at each phonatory level. The canto voice features derive from those of the peripheral nervous system that serve the phonatory organs, in parallel with morpho-functional variations innated and acquired by training. The remarkable properties of recurrent nerves (energy reserve, thickness difference, bi/tri/tetra phase), reflex mechanisms starting from and to the oropharyngeal pavilion (returned impedance, pharyngeal-recurrent reflex) and synapses between origin nuclei of the cranial nerves IX, X, XI, and XII are the basis of special vocal abilities.

This research direction has been materialized by publishing the following articles:

1. Hînganu MV, Hînganu D, Cozma SR, Asimionoaiei-Simionescu C, Scutariu IA, Ionesie DS, Haba D. Morphofunctional evaluation of buccopharyngeal space using three-dimensional cone-beam computed tomography (3D-CBCT). Ann Anat 2018; 220: 1–8;
2. Hinganu MV, Cozma RS, Ciochina P, Scutariu IA, Asimionoaiei-Simionescu C, Hinganu D. The morphometry of the laryngeal phonatory system – base of the anatomical study of the voice aptitudes. Rom J Morphol Embryol 2017; 58(4): 1365-1369.

3.1. Introduction and conceptual backgound

Speaking is one of the characteristics of the human race and the main factor that has marked our progress over time. The singing voice is the crowning of the speech act and the main component of the lyrical manifestation of personality. Doctors in various fields, but especially anatomists have been concerned about discovering how the voice and the substrate of its variability are formed, but these aspects have not yet been fully deciphered.

The studies that I will present are the starting point in our research on the phonatorry system, organized on three levels: laryngeal, oral, palatinal, pharyngeal, epiglottal and nasal.

We performed the dissection of seven embalmed anatomical parts, on which, we made measurements of the anatomical elements involved in the phonation. We performed the same measurements on a batch of seven adults investigated by magnetic resonance imaging (MRI). The results were entered into the statistical calculation formulas and compared with each other and with the literature. The results of the study show that certain values resulting from the calculation formulas remain constant and others vary greatly from each individuals and gender.

We have also evaluated the anatomical functional changes of the buccopharyngeal space in the case of canto voice. The interest in this field is particularly important in view of the relation between the artistic performance level, phoniatrics and functional anatomy, as voice formation mechanism is not completely known yet. We conducted a morphometric study on three soprano voices that differ in type and training level. The anatomical soft structures from the superior vocal formant of each soprano were measured on images captured using the Cone-beam Computed Tomography **(**CBCT) technique.

The results obtained, as well as the 3D reconstructions emphasize the particularities of the individual morphological features, especially in the case of the experienced soprano soloist, which are found to be different for each anatomical soft structure, as well as for their integrity. The experimental results are encouraging and suggest further development of this study on soprano voices and also on other types of opera voices.

3.2. Radiologic anatomy of singing voice

The opera voice has always attracted the interest of researchers from both the artistic and medical field.

This paper reveals the preliminary results of the project called "*Morphofunctional Study of the Vocal Superior Formant*". The protocol for this study was endorsed by the Scientific Research Committee and Research Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi on 20.03.2018. In this study we aim to identify the anatomical and functional changes of the buccopharyngeal pavilion in soprano voices. The pre-existing vocal skills have been evaluated by canto specialists, for sopranos with different levels of training.

The existing literature presents the phonatory apparatus as being composed of three floors (formants), namely: infraglottic, infralaryngeal - determining the amount and pressure of the air column coming from the lungs; the glottic floor of the larynx - producing the so called fundamental frequency of the voice; the ensemble formed by the supraglottic, buccopharyngeal and nasal formants – which are modulating the voice.

The infraglottic floor of the larynx extends from the lower margin of the cricoid cartilage to the inferior arcuate line of the vocal cords (Kutta et al., 2006).

Glottic floor of the larynx extends from the arcuate line of the vocal cords to the horizontal plane through the lateral margin of the Morgagni's sinus (Mor and Blitzer, 2015). It is located between anterior and posterior commissures of the vocal cords (Paulsen and Tillman, 1997; Tillman and Paulsen, 1995).

The superior limit of the supraglottic floor is represented by the superior surfaces of the epiglottis and aryepiglottic folds (Mor and Blitzer, 2015). Together with the oral cavity and oropharynx, it forms the superior vocal formant (Berkeand Long, 2009).

The amount and pressure of the air column on the infraglottic formant depends on the shape and dimensions of the thorax, phrenic muscles and morphological characteristics of the superior and inferior respiratory tract. The entire air column is fragmentarily released to the glottic floor of the larynx by the contraction and relaxation of the muscles attached to the true vocal folds.

The superior formant is the one that modulates the fundamental frequency, rendering the final voice. This modulation is achieved in three main ways: adding of harmonics through the vibration of fibromuscular components existing at this level; absorption of a certain amount of sound vibration; creating a returned impedance from the palatinal and pharyngeal level, which is stimulating the reflex zones of both the bulbar nuclei of the vagus and recurrent laryngeal nerves. This phenomenon allows the recurrent nerves to split their fascicles or even to divide them into 3 or 4 bundles of fibers.

Returned impedance is created by stimulating certain reflexogenic areas at the palatinal and pharyngeal level and also by guiding the main airflow towards these pressoreceptors (Husson, 1960).

These three phenomena occurring at the supraglottic, supralaryngeal floor of the phonatory apparatus are the ones rendering the nature and final quality of the voice. The ability to produce returned impedance is an individual feature that depends on an "antenna" type mechanism.

This antenna transforms the radio currents of the air flow into electric currents. According to this model, the entire subjacent phonatory apparatus constitutes the emitter, while the receiver is represented by the soft palate, palatine veil with its pillars and the oropharynx.

At the receptor level, there is a precise metameric arrangement of its subunits (pressoreceptors of the glossopharyngeal nerve), distributed according to the level of the sound waves to which they are sensitive. Thus, their distribution on the velopalatine mucosa is from the anterior towards the posterior, starting from a stimulation threshold of about 1000 decibels to about 3500 decibels (Sundberg, 1988; Voss et al., 2018).

The present research is an anatomo-imagistic study, which aims to highlight the morphofunctional characteristics of the "antenna apparatus", in order to establish the intrinsic factors that actively and passively participate in the formation of the soprano voice.

This type of study is highly applicable in different fields of activity, such as: medicine, music and scientific.

3.2.1. Material and Methods

The study was conducted on a group of subjects who have signed the agreement concerning this investigation. The study was performed in accordance with the current laws and written consent of the Scientific Ethic Committee of the Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania. It is very important to specify that this study considered adult subjects with no pre-existing pathological conditions that

would influence their voice quality. This will be certified on the basis of the medical history and clinical examination.

Each person was subjected to CBCT investigations both when expressing basic vowels in phonetics, such as "I, Ə:" (called phonatory state I and phonatory state I) and in mimed phonation. When pronouncing the vowel "I" the larynx is in the highest position, while when pronouncing the vowel "Ə" the larynx goes to its lowest position.

The entire investigation took 15 minutes, with an exposure duration of 42 seconds.

The group of subjects, studied and examined for this preliminary report, consisted of three persons, with phonatory characteristics of the soprano voice assessed by canto teachers. The first subject (S1, 22 years old) has a medium vocal practice level (7 years) while the third one (S3, 48 years old) is an experienced soprano soloist having over 20 years of practice. The second subject (S2, 44 years old) has also vocal features of soprano, but with a vocal practice of over 15 years in the theater. It must be mentioned that the theater voice requires vocal exercises similarly to the opera voice, as well as vocal techniques suitable to the profession of actor.

This initial selection of the study group is based on the fundamental principle according to which function dictates the shape and vice versa. Any anatomical structure undergoes shape changes adapted to the intensity of the functional demand. In this regard, an anatomical variation owned by the subjects with special vocal abilities will be more evident in people with a long vocal practice.

The common morphometric characteristics of the studied subjects will be considered anatomical variations that empower the person concerned for vocal skills of canto.

Following the CBCT investigations, the mobile anatomical structures involved in the delimitation of the superior vocal formant (the palatine veil, palatine veil pillars, epiglottis, valleculae, base of the tongue and the oropharynx) were measured, marking their position in relation to the skeletal projection and determining the distances between the muscular and ligamentary structures.

- ***Description of the imaging technique***

Cone-beam Computed Tomography (CBCT) is a state-of-the-art imaging technology that allows the 3D viewing of the scanned areas.

The procedure has a short duration, and the degree of exposure to radiation in a computed tomography performed with CBCT is equivalent to a fully radiological dental status(Chan et al., 2010; Scarfe et al., 2008; Arai et al., 1999). The 3D conical beam technology has reached new levels and has became the reference point in maxillofacial imaging but has never been used to explore vocal training and modulation mechanisms.

According to existing literature, the spatial resolution of CBCT is higher than the one of CT (Scarfe et al., 2008; Al-Ekrish and Ekram, 2011; Kobayashi et al., 2004; Suomalainen et al., 2009; Loubele et al., 2009; Naitoh et al., 2009; Mah et al., 2010; Nomura et al., 2010).

The great advantages of the CBCT equipment are given by the increased flexibility (these devices having an exploration protocol that includes different sizes, as well as height and diameter, depending on the clinical utility), accessibility, easy handling and accurate information from multi-plane and three-dimensional reconstructions obtained from a single, low dose irradiation exam(Ludlow et al., 2008; Loubele et al., 2009; Roberts et al., 2009;

Hein et al., 2002; Ruivo et al., 2009; De Cock et al., 2012; Liedke et al., 2009; Lofthag-Hansen et al., 2011; Razi et al., 2014).

➢ ***CBCT examination protocol***

The CBCT equipment used was Planmeca Promax 3D Mid (Planmeca OY, Helsinki, Finland). Scanning was performed by selecting a 20 x 17 mm FOV with the following exposure parameters: 65 kV, 8 mA, 13.9 seconds and 0.4 x 0.4 x 0.4 mm voxel size. Data was processed using Romexis 4.4.2 software. Scanning is done by exposing the seated patient to the equipment. Both the x-ray source and sensor will make a complete cycle of rotation around the cranial-cervical region, resulting in a volume acquisition that is further used for the required reconstructions. Each patient signed an informed consent explaining the purpose of the study.

All the research team has been involved in establishing the appropriate protocol. After repeated previews, a pre-set exploration protocol for 10.2 cm section was selected. The selection of the protocol is justified by the possibility of obtaining images of the buccopharyngeal pavilion from glottis to hard palate, as well as anterior-posterior images from the dental arches to the vertebral column. The inclusion of vertebral landmarks in the CBCT sections is essential because it represents the main fixed reference for the skeletal projection or from where linear measurements begin.

The data processing software enabled the 3D reconstruction of this space and to compare the features of the new model with the results of the linear measurements made in the 2D sections.

The acquired data were interpreted starting from the measured values of the CBCT exploration of the subjects in mimed phonation. Practically, the following measurements were considered (Figure 3.1.):

- the maximum opening of the oropharyngeal isthmus between the palatoglossus (DPG) and between palatopharyngeus muscles (the height of Passavant ridge - DPF);
- the distance between the top of the epiglottis and the body of the hyoid bone (DEH), the base of the tongue (DEL) and the vertebral column (DECV);
- the skeletotopic projection of the glottis (GP);
- the maximum sagittal (ASMV) and axial amplitude (AAMV) of valleculae;
- the position of the palatine veil (PVP) and the skeletotopic projection of its free edge (VPP).

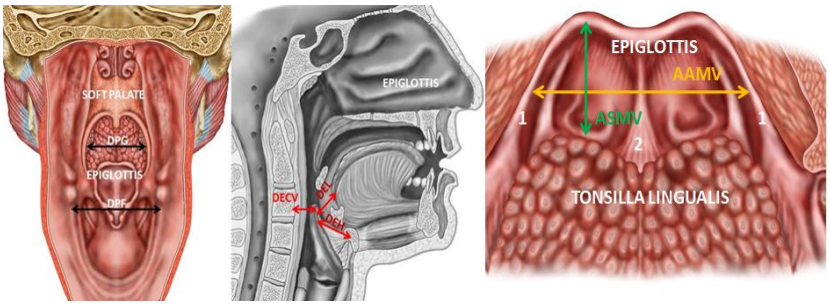

Figure 3.1. Measurements of buccopharyngeal pavilion.1=plica glossoepiglottica lateralis, 2=plica glossoepiglottica medialis

DPG and DPF values characterizes last sphincterlike mechanism of the phonatory apparatus. They are related to functional state of palatoglossus and palatopharyngeus muscles. The contraction of these muscles is changing and permanently adapting the oropharyngeal isthmus to the singer's needs.

The position of the epiglottis related to the base of the tongue characterizes its orientation.

Together with glottic projection, the skeletotopic projection of the epiglottis is a mark of the laryngeal elevation during phonation.

The position of the epiglottis in relation to the hyoid bone describes the complex activity of its muscular apparatus during phonation.

By measuring the vallecular spaces in different phonatory states, we highlight their possible contribution to voice formation.

The skeletotopic projection and position of the palatine veil mark the direction of the airflow towards the anterior opening of the oral cavity and/or the nasal and paranasal cavities.

3.2.2. Results

The results of the measurements are indicated separately for the 3 subjects in each of the three states listed above (MLH - maximum Laryngeal height – or mLH - minimum laryngeal height - and in mimed phonation - MP).

All linear measurements are presented in Tables I-IX.

The data are used for comparison purposes between all three subjects and the existing differences are presented below relative to control values.

The 3D reconstructions performed on the three subjects show significant differences in axial and coronal diameters for the same phonatory state, but also individual differences of these diameters for the measurements made in different phonatory states for the same subject (Figures 3.2.a., b., c., 3.3.a., b., c. and 3.4.).The axial sections at the interest region level can also be colored so that the color of each region depends on its size.

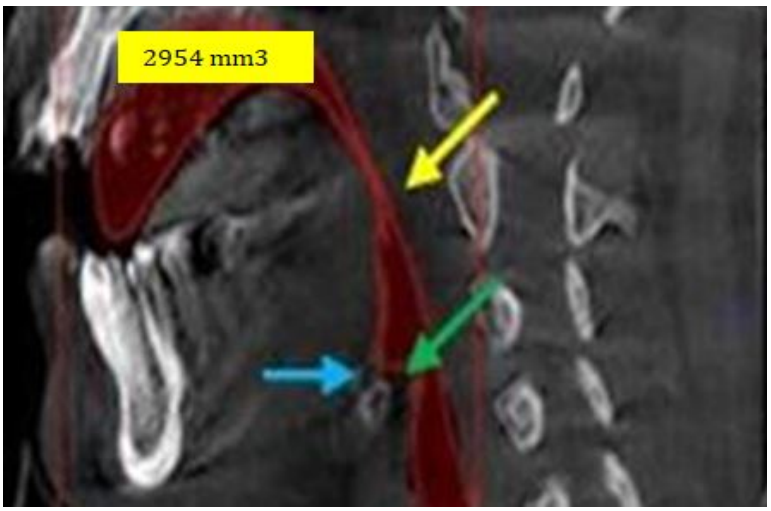

Figure 3.2.a. 3D reconstruction of buccopharyngeal pavilion of subject S1 - phonation with larynx in uppermost position.

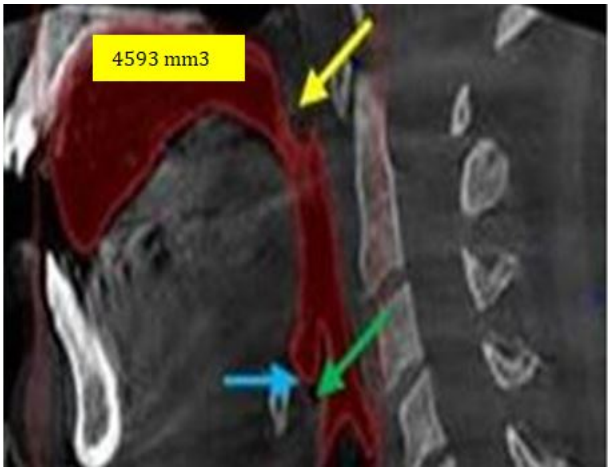

Figure 3.2.b. 3D reconstruction of buccopharyngeal pavilion of subject S1 - phonation with larynx in lowermost position.

Figure 3.2.c 3D reconstruction of buccopharyngeal pavilion of subject S1- miming phonation.

All the 3D reconstructions from Figures 3.2.a., b. and c.are represented in sagittal view. Yellow arrows mark the free edges of the palatine veil; green arrows mark the top of the epiglottic cartilage; light blue arrows mark the valleculae.

All 3D reconstructions from figures 3.3.a., b. and c. are represented in sagittal view. Yellow arrows mark the free edges of the palatine veil; green arrows mark the top of the epiglottic cartilage; light blue arrows mark the valleculae.

All 3D reconstructions are represented in sagital view. Yellow arrows mark the free edges of the palatine veil; green arrows mark the top of the epiglottic cartilage; light blue arrows mark the valleculae.

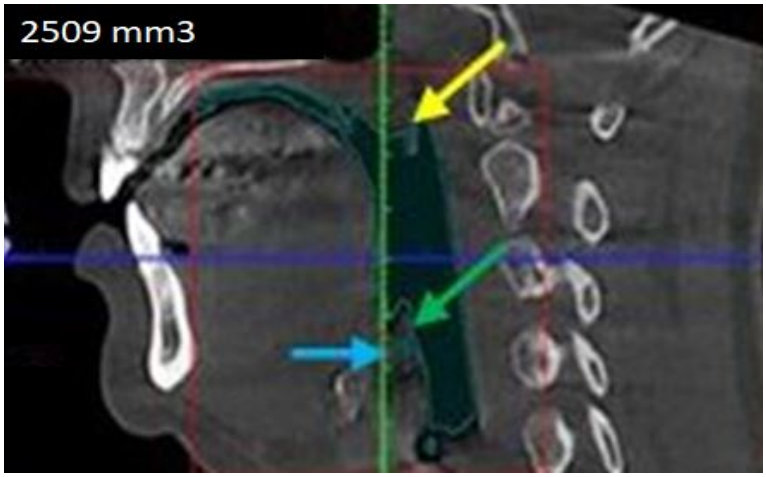

Figure 3.3.a. 3D reconstruction of buccopharyngeal pavilion of subject S2 - phonation with larynx in uppermost position

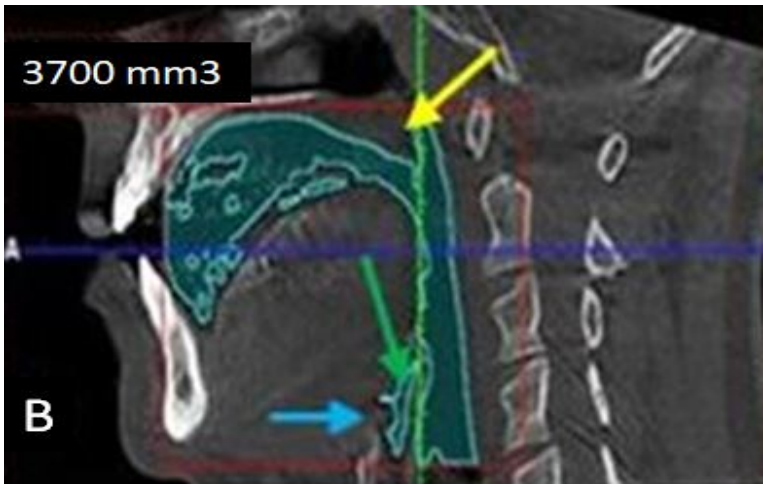

Figure 3.3.b. 3D reconstruction of buccopharyngeal pavilion of subject S2 - phonation with larynx in lowermost position

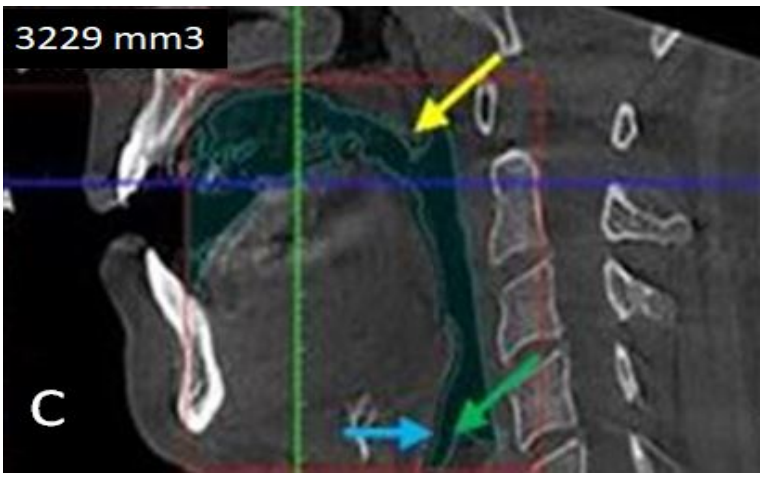

Figure 3.3.c. 3D reconstruction of buccopharyngeal pavilion of subject S2 - miming phonation.

Figre 3.4.a. 3D reconstruction of buccopharyngeal pavilion for subject S3 - phonation with larynx in lowermost position

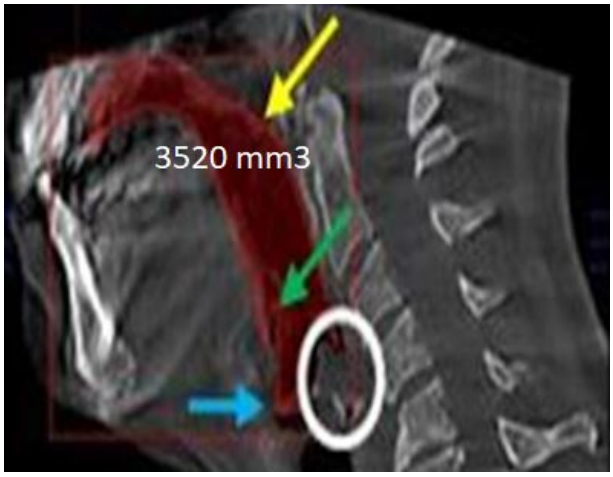

Figure 3.4.b. 3D reconstruction of buccopharyngeal pavilion for subject S3 - phonation with larynx in uppermost position

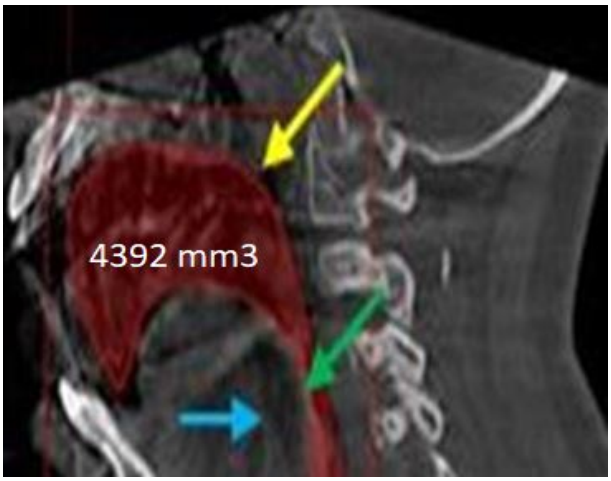

Figure 3.4. 3D reconstruction of buccopharyngeal pavilion for subject S3 - miming phonation; the white circle marks the anterior protrusion of the posterior pharyngeal wall due to the contraction of palatopharyngeus muscles (Passavant's ridge).

The visualization of the 3D reconstructions shows the influence of the buccopharyngeal muscles (palatoglossus, palatopharyngeus, hyoglossus, genioglossus and longus capitis muscles) on the volume and especially on the shape of the buccopharyngeal pavilion in sustained phonation (Figure 3.5.)

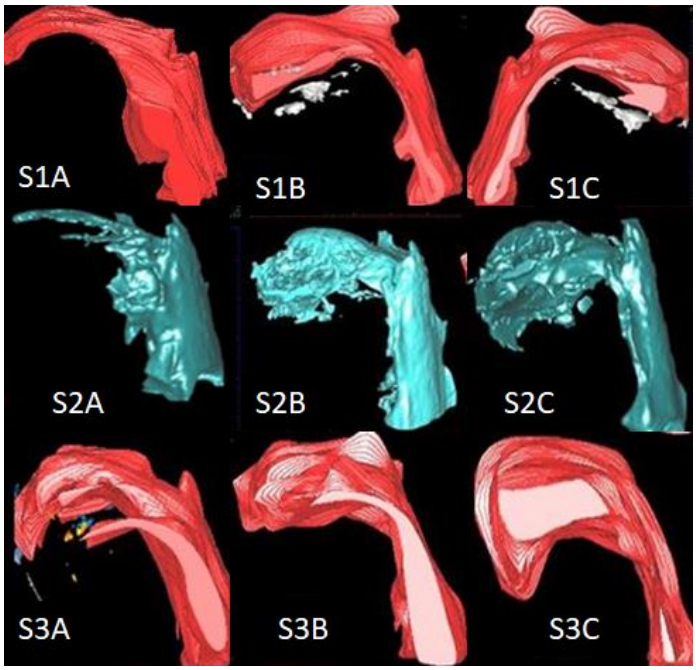

Figure 3.5. 3D reconstruction posterior lateral view; S1A, S1B and S1C - 3D reconstruction of S1 subject while phonating letters "I:", "Ə" and miming phonation; S2A, S2B and S2C - 3D reconstruction of S2 subject while phonating letters "I:", "Ə" and miming phonation; S3A, S3B and S3C - 3D reconstruction of S3 subject while phonating letters "I:", "Ə" and miming phonation.

- *DPG value*
  - For subject S1, in comparison to the value measured in the mimed phonation, this distance decreases in phonation with the larynx in state I and increases in state II, between -17.14% and +14.3%. This does not happen in the case of the other two subjects, where the DPG value decreases in both states (13.9% and 18.4% for subject S2, respectively 45.3% and 4.7% for subject S3) (Table I).
- *DPF value*
  - The values of this measurement are very different from the previous ones, so that in the case of subject S2 they are smaller in both phonatory situations than in mimed phonation but the differences are slightly insignificant (-0.62% and -2.9%) (Table I).
- *DEH value*
  - This measurement was performed in order to evaluate the position of the epiglottis to the hyoid bone (mobile point), which indirectly reveals the interaction of the hyoidian suspensory muscular apparatus in the act of phonation.

- Subject S1 presents DEH values higher than the reference value corresponding to the mimed phonation by 22.83% in the phonatory state I and 12% in the phonatory state II.
- For subject S2, the values are lower than the control value (mimed phonation) in both phonatory states: 41.69% and 22.43%, respectively.
- In comparison to the control value, subject S3 presents in phonatory state II a decreased DEF value with 25.75% and in the phonatory state I a value increased with 12.63% (Figures 3.2.a., b., c., 3.3.a., b., c., 3.4., Table I).

➢ *DEL value*

- In phonatory state I and II, all three subjects present increased DEL values in reference to the control value, as follows: S1 with 123.68% and 23.6%, S2 with 64.17% and 14.08% and S3 with 19.80% and 66.39% (Figures 3.2, 3.3, 3.4, Table I).

➢ *DECV value*

- This measurement reveals that subject S1 has an increase of 22.22% compared to the reference value in phonatory state I and a decrease of 12.17% in phonatory state II. For subjects S2 and S3, these values increase in both phonatory states by 81.45% and 42.11% respectively by 96.20% and 64.00% (Figures 3.2.a., b. and c., 3.3.a., b. and c., 3.4., and Table I).

➢ *GP value*

- The skeletotopic projection of the glottis for subject S1 reveals it to be more elevated in the phonatory state II and more lowered in phonatory state I.
- Subject S2 presents the same glottic projection in the phonatory state II as in the mimed phonation and a lower one in phonatory state I.
- Subject S3 presents a glottic projection somewhat similar to subject S1 (elevated in the phonatory state I and lowered in phonatory state II), but the glottic elevation in phonatory states is projected with an extra vertebra above (Figures 3.2.a., b. and c., 3.3.a., b. and c., 3.4., and Table I).

➢ *ASMV value*

- For this measurement, we recorded higher values for all subjects in both phonatory states.
- Subjects S1 and S2 show a significant increase of the ASMV value in phonatory state I and significant decrease in phonatory state II (48.43% and 8.15% for S1, 76.53% and 23.23% for S2).
- Subject S3 shows a slight increase in the sagittal diameter of the glottis (11.98% and 20.37%), inversely proportional to the other 2 subjects: the ASMV growth values in the phonatory state II are approximately double in reference to the state I (Figures 3.2.a., b. and c., 3.3.a., b. and c., 3.4., and Table I).

➢ *AAMV value*

- The measurement of the transversal diameter of the valleculae has a completely different presentation in all three subjects.

- Subject S1 presents an increase in this diameter in both phonatory states by 18.66%, respectively 27.94%.
- Subject S2 presents a decrease of this value in both phonatory states by 5.85%, respectively 6.15%.
- Subject S3 presents a decrease of the AAMV value by 12.09% in phonatory state I and an increase by 18.17% in phonatory state II (Figures 3.2.a., b. and c., 3.3.a., b. and c., 3.4., and Table I).

➢ *PVP and VPP values*

- The analysis of the position of the palatine veil and the skeletotopic projection of its free edge reveals that, in the case of subject S1, it is almost horizontal in phonatory state I and in mimed phonation and obliquely descended in phonatory state II.
- Subject S2 presents a horizontal palatine veil in phonatory state I and an oblique one in the other two situations. In phonatory state II, its position is lower than in mimed phonation.
- Subject S3 presents a horizontal position of the palatine veil in all three situations, slightly lowered in phonatory state I (Figures 3.2.a., b. and c., 3.3.a., b. and c., 3.4., and Table I).

3.2.3. Discussions

The review of the literature (data bases sources as Clarivate, PubMed, Scopus, Index Copernicus or specialty books) refers to radiologic explorations of the vocal tract, especially MRI (Clément et al., 2007).

None of the explored subjects were related to aging phenomena, which could influence the quality of voice production (Claassen et al., 2009).

In sustained phonation, superior vocal formant acts as a resonator, modifies the signal spectrum and defines both the vocal quality and the characteristics of the voice timber. These aspects are important mainly for opera voices. The semi-automatic segmentation algorithm that works with reoriented images has been used for the 3D reconstruction of the superior vocal formant (Mainka et al., 2017). These data can be used to deepen the understanding of the physiology of the superior vocal formant.

Other related MRI studies describe inaccurate 3D reconstructions possibilities due to supine position of the subject in the MRI scanner, which limits the movements of the hypopharynx (Clément et al., 2007; Kitamura et al., 2005).

Studies that used three-dimensional computed tomography have been conducted on cricothyroid space, during sustained phonation. Authors showed there are two important movements of the cricothyroid joint during high-pitch phonation which occur simultaneously: rotational and anterior gliding movement. Because of these movements, the vocal cords elongate (Hiramatsu et al., 2012). In this study, the authors state that the anterior horizontal gliding is the main movement, leading to the efficient elongation of the vocal cords.

To our knowledge, no studies derived from 3D-CBCT data on the morphology of the superior vocal formant during phonation have been reported so far. We conducted this study

in order to reveal the morphometry of this space under different phonatory states. We did the interpretation of the obtained data for each subject in part, as well as comparatively in the same phonatory states.

Regarding the DPG and DPF values, for subject S3 there is a significantly increased variation of 40.6% of DPG in the two phonatory states, close to the 31.44% value found for subject S1. However, in the case of experienced soprano (S3) this variation is totally negative.

Table I 3D CBCT measurements of the oropharyngeal parameters; DPG=the maximum opening of the oropharyngeal isthmus between the palatoglossus and DPF=between palatopharyngeus muscles; DEH=the distance between the top of the epiglottis and the body of the hyoid bone; DEL=the base of the tongue and DECV=the vertebral column; GP=the skeletotopic projection of the glottis; ASMV=the maximum sagittal and AAMV=axial amplitude of valleculae; PVP=the position of the palatine veil and VPP=the skeletotopic projection of its free edge.

DPG VALUES			
Subject	**MLH**	**mLH**	**MP**
S1	**34.80mm**	**48.01mm**	**42.00mm**
S2	**39.25mm**	**37.22mm**	**45.61mm**
S3	**23.42mm**	**40.80mm**	**42.82mm**
DPF VALUES			
Subject	**MLH**	**mLH**	**MP**
S1	**35.21mm**	**35.60mm**	**30.04mm**
S2	**33.61mm**	**33.65mm**	**33.82mm**
S3	**36.51mm**	**47.18mm**	**39.40mm**
DEH VALUES			
Subject	**MLH**	**mLH**	**MP**
S1	**21.20mm**	**19.33mm**	**17.26mm**
S2	**15.01mm**	**19.97mm**	**25.74mm**
S3	**26.08mm**	**18.12mm**	**20.74mm**
DEL VALUES			
Subject	**MLH**	**mLH**	**MP**
S1	**15.21mm**	**8.41mm**	**6.80mm**
S2	**9.21mm**	**6.40mm**	**5.61mm**
S3	**7.20mm**	**10.00mm**	**6.01mm**
DECV VALUES			
Subject	**MLH**	**mLH**	**MP**
S1	**22.00mm**	**15.81mm**	**18.00mm**
S2	**27.58mm**	**21.60mm**	**15.20mm**
S3	**19.62mm**	**16.40mm**	**10.00mm**
GP VALUES			
Subject	**MLH**	**mLH**	**MP**
S1	**C6**	**C7 lower 1/3**	**C7 upper 1/3**
S2	**C6–C7 intervertebral disc**	**C6**	**C5–C6 intervertebral disc**
S3	**C5–C6**	**T1**	**C7–T1 intervertebral disc**
ASMV VALUES			
Subject	**MLH**	**mLH**	**MP**
S1	**18.39**	**13.40**	**12.39**
S2	**21.20**	**14.80**	**12.01**
S3	**21.97**	**23.61**	**19.62**
AAMV VALUES			
Subject	**MLH**	**mLH**	**MP**
S1	**34.40mm**	**37.09mm**	**28.99mm**
S2	**30.92mm**	**30.82mm**	**32.84mm**
S3	**32.80mm**	**44.09mm**	**37.31mm**
PVP and VPP values			
Subject	**MLH**	**mLH**	**MP**
S1	**C2 horizontally**	**C3 – obliquely**	**C1–C2 – horizontally**
S2	**C1–C2 horizontally**	**C2–C3 obliquely**	**C2 – obliquely**
S3	**C2 – horizontally**	**C1 – horizontally**	**C1 – horizontally**

Subject S2 has a variation of only 4.5% between the two phonatory situations.

Both values are higher in phonation for subject S1: +4.12% and +18.51%.

For subject S3, the values in phonation are diametrically opposed to the control value (mimed phonation): - 7.34% and +19.75%.

When comparing the DPG and DPF values for subject S1, it results that the oropharyngeal isthmus narrows from the posterior to the anterior in phonatory state I and opens anteriorly in phonatory state II.

In the same way, in the case of subject S3, the opening of the isthmus will be backwards, similar to subject S1: the posterior diameter decreases by 37.96% in reference to the anterior one in phonatory state I. In phonatory state II happens the same situation but the values are higher.

For the anterior opening of the isthmus, subject S2 has negative relative values of 13.28% and 15.5% in the two phonatory states.

The three subjects are totally different in the two phonatory states. The greatest differences are in the case of the experienced soprano, where the morphology and the phonatory role of the oropharyngeal isthmus are different (wider openning in phonatory state II).

The DPG value characterizes the implication of the palatoglossus muscle into the voice formation. Studies about this muscles show they may receive branches from the trigeminal nerve, which explains its participation in the formation of the velopharyngeal impedance (resistance) during phonation (Abd-El-Malek, 1938; Fehrenbach, 1998; Moore, 2014).

The contraction of palatopharyngeus muscles could produce a mucosae fold called Passavant's ridge and participates to the anterior movement of the posterior wall of the oropharynx (Calcan, 1958;Yamawaki et al., 1994; Yamawaki et al., 1996).

The interpretation of DEH, DEL and DECV results reveals that subject S1 produces an anterior protrusion of the oral floor in both phonatory states, subject S2 a retropulsion, and S3 produces a retropulsion in state I and a protrusion in state II. It was possible to correlate the movements of the oral cavity floor with the elevation of the base of the tongue. Acute sounds involve higher elevations of the oral floor.

The values of this measurement present specific particularities for the experienced soprano, for which the increases are in inverse relation to the other two subjects - the DEL value increases much more in the phonatory state II than in state I. This means that the first two subjects produce a greater anterior protrusion of the tongue in the phonatory state I than in II while subject S3 behaves diametrically opposed.

We emphasize that this measurement shows that subject S3 has the highest increase of these values in both phonatory states.

By comparing the GP values for all three subjects, it can be concluded that the experienced soprano has the maximum laryngeal height in phonatory state I (acute vowels).

The elevation of the larynx contributes to the elongation of the vocal cords which stimulates vocal muscles contractions. The particular arrangement of the vocal muscle insertions allows them to accelerate their contraction rate by two mechanisms: individual characteristics of the recurrent laryngeal nerves which can split their fascicle in two, three or even four bundles and tension receptors of the vocal ligaments (Kutta, 2007).

Measurements of the ASMV and AAMV values show that, in opposition to the other two subjects, the experienced soprano soloist achieves a larger amplification of the length of valleculae in the phonatory state II than in the first one.

Analysis of the ASMV and AAMV measurements indirectly indicates volumetric changes occurring at the valleculae level in the singing voice. In relation to this situation, subject S1 presents an increase of the sagittal and transversal diameters in both phonatory states. Subject S2 has increased sagittal diameters in both phonatory states, but partially compensates by decreases in the transversal ones.

In the case of the experienced soprano, the increase of the sagittal diameter in phonatory state I is compensated almost entirely by the decrease of the transversal diameter. In the phonatory state II, both diameters increase.

Thus, we can conclude that subjects S1 and S2 present apertures of vallecular spaces in both phonatory states, while for subject S3 this happens only in phonatory state II.

Related literature describes epiglottic vallecula as "spit traps" which prevent the initiation of the swallowing reflex (Briche et al., 1991) but no data are available about its possible function in phonation.

After analyzing the PVP and VPP values, we can conclude that subjects S1 and S2 are nasal in the phonatory state II while the experienced soprano is nasal in none of the states.

All these values are characterizing and directly influencing the velopharyngeal space. The velopharynx (VP) is a complex muscular sphincter, influencing acoustic and aerodynamic energy through oral and nasal cavities. The movements of its muscular walls alter the size of this sphincter and give him the special functional adaptability (Barlow and Stumm, 2009).

3.2.4. Conclusions

Using CBCT in anatomical exploration of the superior vocal formant is a new and complex method. Choosing the right imaging protocol that considers the entire explored area and fixed marks is the key to this functional radiological investigation. The ability to accurately measure soft structures and the spaces between them with minimal exposure to X-rays make CBCT a highly performing and useful exploration. Making the 3D reconstructions brings for the first time information about the changes that take place during phonation at the buccopharyngeal pavilion.

3.3. The morphometric evaluation of the phonatory system

Speaking characterizes the human race and explains the possibility of communication and transmission of information presented in most people. The singing voice is the artistic form of speech and a peculiarity of individualexpression. This is addressed to ”talented”people who can do it in a pleasant way for the general public. This study is intended to be the beginning of a research of this type of talent and a modality of qualitative and quantitative appreciation of it. The origin of the sound is found at the specialized structures of the laryngeal glottal floor. The fundamental frequency of a voice is conditioned, first of all, by

the individual anatomical features of the phonatory laryngeal system. In this study, we highlighted the anatomical variations, but also the constants of the laryngeal phonator system by using modern imaging methods.

Communication is an essential process of maintaining human relations, being the foundation of social organi-zation. The branch of medicine dealing with the study of voice, illness and voice disorders is phoniatry. The *European Union of Medical Specialists*(UEMS) classifiedit, in 2010, as one of the seven subspecialties of otorhino-laryngology (Oguz 2015).

Phonation requires precise coordination of breathing, control of the specialized laryngeal structures involved in this process, as well as a control regarding the positioning of the tongue, lips and mandible (Seikel, 2009).

Speech is based on the formation of complex sounds, a combination of fundamental sound and a rich system of harmonics (Standring, 2016).

The main objectives of our study are:

- identification of a spectrum of anatomical variations of the laryngeal phonatory system from different individuals;
- the relation between the anatomical structures and their morphometry on the fundamental frequency of the voice;
- highlighting the particularities of the structures responsible for the mechanism of laryngeal sound production.

The first step in this direction is exploring the laryngeal floor of the phonator system.

3.3.1. Materials and Methods

The study was conducted on a number of seven anatomical parts preserved by formolization, at the "Ion Iancu"Institute of Anatomy of the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania. On a group of seven adults, the laryngoscopical examination was first performed. We followed the macroscopic appearance of the larynx and the dynamics of the vocal cords, in order to exclude the persons with associated pathology of phonator apparatus. The subjects stated that they do not know having a laryngeal pathology and do not accuse dysphonia or other voice changes. At the same time, they deny smoking, a habit that affects the larynx, influencing the results. In the second step, accurate measurements were performed on magnetic resonance imaging (MRI) images, which were used in different formulas.

We dissected and highlighted the cartilaginous, muscular, and ligamentous structures of the larynx, then we made measurements at this level. The values obtained were used in statistical calculation formulas.

In the macroscopic, clinical and radiological anatomystudy, a statistic was performed by using the metric values of the laryngeal ligamentous apparatus involved in the phonation and the volumetric values of the infraglottal filter (volume of the infraglottal floor).

3.3.2. Results

Measurements were made on: DAP: Anteroposterior diameter of glottis; GMD: Glottal maximum diameter; VCL: Vocal cords length before and after mucosal removal; DC: Maximum distance between vocal cords; ITL: Interarytenoidian distance. HIG: Height of infra-glottal floor; VIG: Volume of infraglottal floor, and MIG: the length between the voice processes (Figures 3.6.–3.9.) on anatomical specimens and also on MRI images (Figures 3.10. and 3.11.).

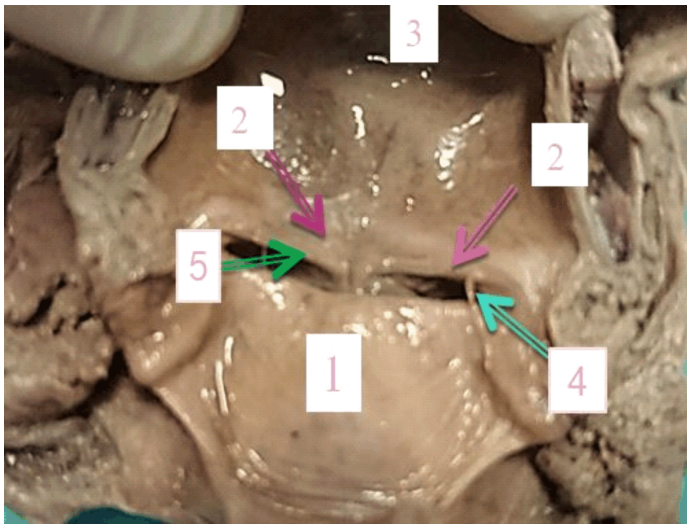

Figure 3.6. – *Dissection piece. 1: Supraglottal floor; 2: True vocal folds; 3: Infraglottal floor; 4: False vocal folds; 5: Laryngeal vestibule.*

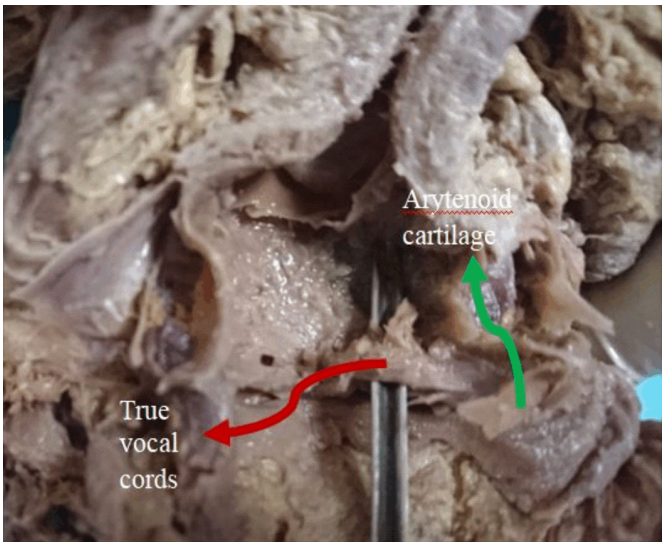

Figure 3.7. – *With red arrow, true vocal cords; with green arrow, arytenoid cartilage.*

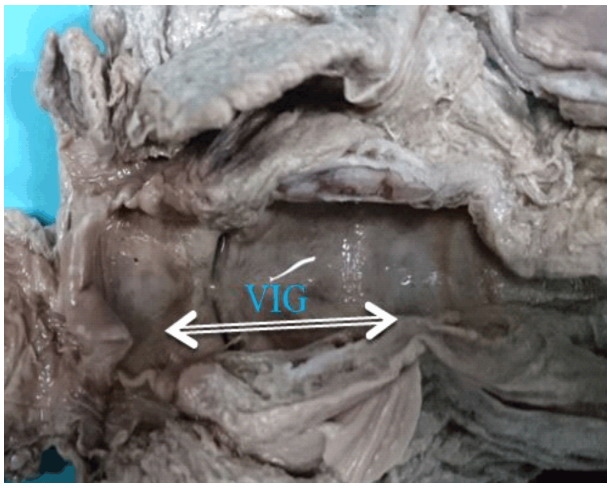

Figure 3.8. – *Dissection specimen: measurement of infraglottal volume.*

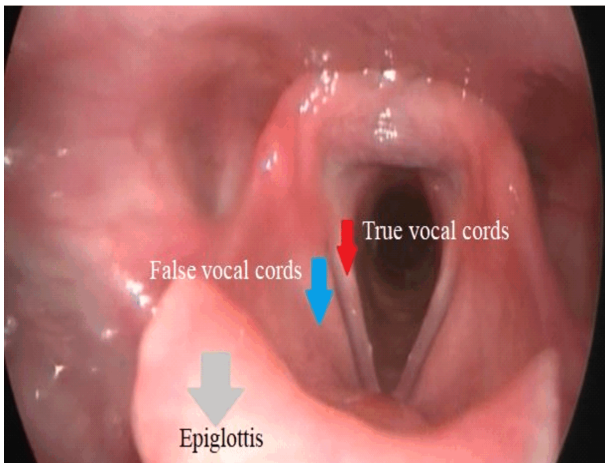

Figure 3.9. – *Laryngoscopic examination: normal, no pathological changes.*

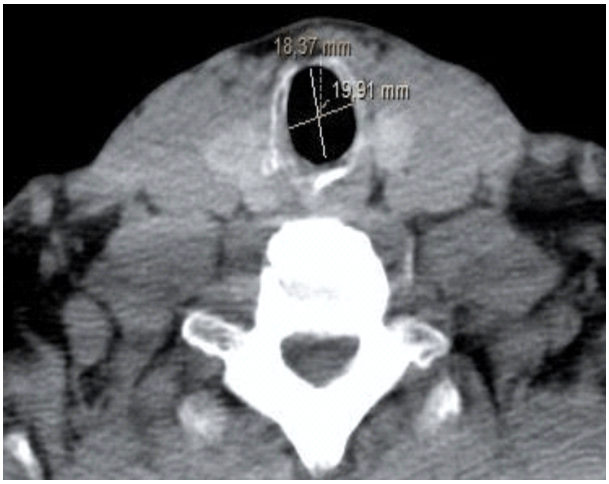

Figure 3.10. – *Forced exhale: vocal cords are getting closer and arytenoid cartilage can be recognized. MRI image.*

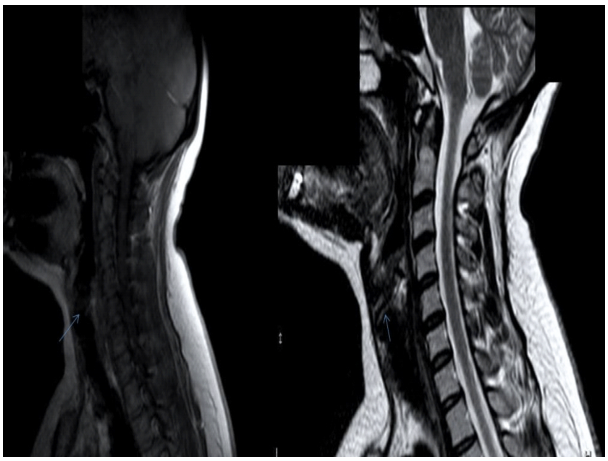

Figure 3.11. – *MRI highlighting of false vocal cords (left) and true vocal cords (right).*

Taking as a reference the average size measured for each structure, we obtained different variations, which for a better understanding we expressed as a percentage.

For measurements on dissection specimens, the DC/VCL ratio has an average value of 2.53 with a variation range of 24.05%. DAP/VCL has an average value of 2.4, with a variation of 11.27%. DAP/ITL has an average of 2.4 and a variation range of 6.4%.

Between male and female gender, the highest morpho-metric differences were found in the VIG (20%), HIG (50%) and the GMD/HIG (3.2%).

Table II – *The results of the measurements on MRI images*

Measured parameters	**Female subjects**		**Male subjects**	
VCL (lengths of vocal cords) [cm]	*Right* 1.49 ±0.11	*Left* 1.46 ±0.07	*Right* 1.68 ±0.08	*Left* 1.64 ±0.1
GCV (thickness of vocal cords) [cm]	*Right* 0.866 ±0.29	*Left* 0.867 ±0.23	*Right* 0.897 ±0.46	*Left* 0.804 ±0.11
HVC (height of vocal cords) [cm]	*Right* 0.5 ±0.32	*Left* 0.502 ±0.28	*Right* 0.735 ±0.32	*Left* 0.608 ±0.45
DAT (arytenoid diameter) [cm]	*Right* 0.346 ±0.14	*Left* 0.307 ±0.23	*Right* 0.62 ±0.4	*Left* 0.69 ±0.65
DAP/GMD (anterior-posterior glottal diameter) [cm]	1.863±1.5		2.55±4.33	
ITL (interarytenoidian distance) [cm]	1.64±0.67		1.33±0.98	
MIG (the length of voice processes) [cm]	1.114±0.84		0.99±1.05	
Cricoid ring diameter [cm]	1.47±1.19		1.99±1.23	
HI (height of infraglottal floor) [cm]	0.996±0.12		1.24±0.85	
Volume of infraglottal floor [mL]	0.988±0.54		1.167±0.8	

The same measurements made by the imaging technique are shown in Tables II and III. Relatively constant ratios between vocal folds and interarytenoid distance, as well as between vocal folds and the maximum opening of vocal processes demonstratethat at the glottis level the measured dimensions increase directly proportional.

Table III – *The results of the measurements on MRI images*

Ratios	**Female subjects**	**Male subjects**
VCL/ITL	0.9	0.125
VCL/VIG	0.014	0.0014
DAP/VCL	1.25	0.154
DAP/ITL	1.13	0.149
GMD/MIG	1.67	2.31
GMD/VIG	0.018	0.016

The ratios obtained between these values are as follows: LCV/VIG=10.48–19.1%; DAP/VCL=4.16–9.38%; DAP/ITL=8.34–12.16%; VCL/VIG=1.9–27.27%; DAP/ VCL=9.09–11.03%; DAP/ITL=10.49–13.37%; VCL/ITL=12.3–16.29%; GMD/MIG=15.91–30.92%; GMD/VIG=12.72–25.04%; VCL/ITL=16.8–29.6%; GMD/MIG=12.12–67.09%; GMD/VIG=6.25–31.25%.

A large interarytenoid distance results in longer vocal folds and a larger antero-posterior glottal diameter.

These findings indicate a complete functional specialization of the glottal structures, from the phonatory point of view.

We have obtained ratios whose results are significantly higher than average, such as VCL/VIG, with a variation of up to 131.57%, where we have a VCL of about 3 cm, given by the fact that the female VCL is about 1.1–1.5 cm. In this case, an inverse proportional variation is observed,as the VCL increases, the VIG decreases, so the amplitude of a sound is not given by VCL, but by its tension.

The GMD/VIG ratio also has high variations of up to 121.42%, but due to the increased VIG. VIG gives us a certain amount of air that we can release gradually or not during phonation, and the bigger it is, the lower is the voice quality – especially for singers. Narrow GMD and a small VCL determine the production of a sound with acute, high, timbre, characteristic of female gender.

A large GMD and an increase in LCV characterize male gender with baritone voice production and the fundamental frequency decreases.

The phonatory adaptation of the glottis is different from that of the height and volume of the infraglottal floor. This can influence the harmonic vibrations of a certain fundamental frequency with a constant infraglottal pressure.

Infraglottal pressure has a modulating role in voice timbre (it influences the pharyngeal-buccal harmonic of the voice) and depends on the infraglottal volume and the type of breathing.

An increased infraglottal volume can be compensated by adequate respiration.

As a result of the measurements, there was a greater variation in a set of ratios. Therefore, there is an inverselyproportional relationship between the parameters of eachincreased ratio. Those values with a variation of less than 5% suggest a direct proportionality relationship (Figure 3.12).

The results obtained were compared with the results ofsimilar measurements made on embalmed pieces. The ratios where major numerical differences were reported are:

- VCL/VIG (18.42–131.57%) – this difference is mainly based on the existence of 3 cm of vocal cords at the level of an embalmed piece;
- GMD/VIG (28.57–121.42%) – the percentage varies due to the high value of VIG (9.55), which may indirectlyindicate an anomaly of the phonator apparatus or the factthat the piece belonged to a person which performed in musical field (increased infraglottal volume allows to maintain musical effort).

This study highlighted the fact that the fundamental frequency is governed by many parameters that can be studied and reported in various forms.

These fractions are aimed at identifying constant or non-constant parametersfrom physiological point of view, with conclusions being drawn from current anatomical functional knowledge. Also, the morphometric study of the normal phonator system is important for differentiation from the pathology associated with it.

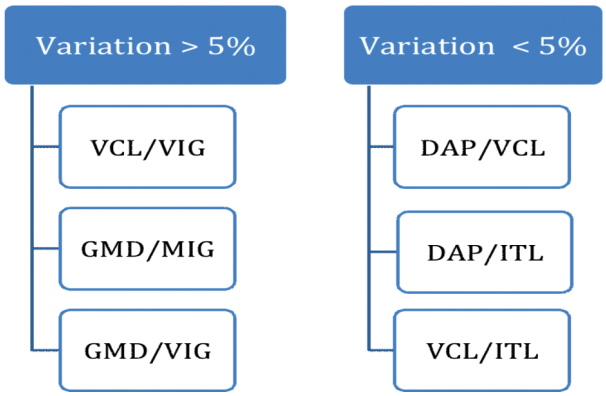

Figure 3.12. – *Values with a variation of less than 5%.*

3.3.3. Discussions

"Sounds are perceived as human vocalizations when they are produced by a vocal system that follows the simple relationship between the size of the vocal folds and vocal tracts. We have found that these anatomical parameters encode the perceptual vocal identity (man, woman, child) and show that brain areas that respond to human speech also encode vocal identity" (Assaneo, 2016).

The data collected by direct laryngeal morphometry on dissected pieces are very close to those in the literature (Joshi, 2015; Kaur, 2014; Patel, 2016) and confirm the substrate of the fundamental differences between the two genders regarding the parti cularities of phonation in adults.

The literature describes three major vocal subsystems that interact with each other: non-linear source-filter interactions, airflow control by glottal adduction, and tracheal-induced vocal tract elongation (Herbst, 2017). The three vocal formants are the infraglottal filter that determines the amount and pressure of the air column coming from the lungs, the glottal floor of the larynx that produces what is called the fundamental frequency of the voice, and the ensemble formed by the supraglottal, oro-velo-pharyngeal and nasal formations, which modulates the voice (Dankbaar, 2014; Muresan, 2013; Ramos, 2015; Ameida 2010).

Measurements performed on MRI images are also very close to those obtained by direct measurements and in accordance with literature data (Schmaulfuss, 2003; Lingala, 2016; Thiagarajan, 2015). However, we have not found in the literature another study to correlate the values obtained from these measurements (direct and imaging) and to produce statistical formulas based on these.

The parameters ratios that we have investigated characterize the so-called vocal quality (a parameter studied in phoniatry, which involves voice control, being defined by a number of factors: vocal cord configuration, laryngeal anatomy, voice control capability. VCL/VIG is highly varied. So, VCL varies inversely proportional with VIG. An increased infraglottal volume is associated with a low vocal cord length, in order to allow the support of the vocal cords vibration, in a single expiration, during the phonation or during the musical act.

GMD/MIG – there is a relationship of inverse proportionality between the two parameters, both anatomically (the vocal processes have an internal oblique distribution,the distance between them versus the maximum diameterof the glottis being lower), and functionally. The distance between the vocal processes, the vocal muscles, can be controlled in the idea of producing and supporting notes of low or high intensity. GMD/VIG – the infraglottal volume value is increased, as compared to the maximum glottal diameter, to allow the passage of fragmented air through the glottal slit in a single expiration. DAP/VCL-DAP is directly proportional to VCL.

The dynamics of the vocal cords follow the anterior-posterior diameter of the glottal slit, these two laryngeal components functioning in harmony for the adduction, respectively the abduction of the vocal cords. DAP/ITL, VCL/ITL – the distance between the vocal processes of the arytenoid cartilage and the anterior-posterior diameter must be symmetrical for aligning the midline of the vocal cords and for completing the phoning process. Increasing the length of the interarytenoid distance is associated within increase in DAP and VCL, respectively, to maintain a phonator or musical effort.

3.3.4. Conclusions

The quality (amplitude and penetrance) and the timbre of a voice vary inversely with the dimensions of the vocal cords and GMD. Their low values are characterized by an acute timbre, and their high values by a gravely timbre. The laryngeal filter, the infraglottal floor, and the sphincter of true vocal folds are responsible for the fundamental frequency with which a sound is produced. We will be able to use these formulas on patients for an indirectly determining of the fundamental frequency of voice in those individuals. The obtained statistical

results encourage us to believe that we have laid the foundations for creating an imaging protocol by which we can determine the characteristics of the fundamental frequency of an individual.

3.4. Further enhancements

Quantification of the anatomical variations of the phonator system is essential in understanding the personality of an individual. Its morphometry is the starting point for studying the harmonic capacities of each individual's voice.

Our pilot study on oropharingeal opening demonstrates the usefulness of CBCT in the study of superior phonatory formant, opening the way for future studies on statistically significant number of subjects. The practical applicability of this research is especially in the study of functional and clinical anatomy of morphology and volumetry of buccopharyngeal space.

Futher research in this field should be done using new exploration techniques such as fMRI in order to mark and trace the centripetal paths of sensitivities triggered by the sound stimulus played at the laryngeal, hypopharyngeal and oral cavity. I propose to perform mucosal and vocal muscles experiments to detect types and subtypes of nerve receptors at that level.

SECTION II – Professional, Scientific and Academic Development

An university career is, in my opinion, the top of professional education. The opportunity to complete it in the academic environment is a great personal achievement that forces me to honor it.

Being a good pedagogue requires a permanent formation and development of the teaching staff. They should be able to give those who teach them the chance to understand the mysteries of the domain they teach, to stimulate their interest, and ultimately to awaken their love for it.

1. Career overview and professional achievements

In 2005 I was admitted as a resident in the specialty of General Surgery by Order of the Minister of Health and in 2011 I finished this specialty, with the title of General Surgery General Practitioner. Starting with 2012 until now, I work at the 2nd Clinic of Surgery of "Sf. Spiridon"with clinical integration.

The experience gained from this side of my professional development has helped me in my teaching career, giving me the opportunity to put into practice what I had learned in theory or through dissection techniques on the cadavers. This aspect of my professional achievement has been another cornerstone in developing and deepening personal knowledge, in understanding the applied anatomy, because "in medicine, you must pass from the cadaver

to the living human and not the other way round." Surgery is dependent on anatomy, as evidenced by the many personalities of Romanian surgery who started their career as anatomists.

Based on this experience, I followed the postgraduate course Digestive Diagnostic Endoscopy and passed the attestation exam for this.

1.2. **Academic activity**

I graduated the Faculty of General Medicine in 2004, and since 2005 I have been taking part in the practical classses of Anatomy, as an Associate Instructor at the Faculty of General Medicine of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi, within the Anatomy Discipline III.

The experience gained in all these years made me realize the importance of getting students to be attracted to classes, raising their interest, primarily by personal example and by emphasizing the practical importance of what they are studying. Organizing and conducting seminar activities within the laboratories we focused on the involvement of students in practical activities and centered on developing their capacity for expression and exposure in an academic way.

My constant preoccupations to increase the value of the didactic act from a methodological point of view were based on:

- ✓ the use of modern teaching techniques, in line with the new trends in university pedagogy, starting with the use of the retro-projector and up to the current virtual dissection videoprojectors and tables. With the help of these I taught the students exemplifying with the images acquired in the dissections performed personally and on the synthesis from courses bibliography;
- ✓ highlighting and discussing the history of the university and, in particular, the Institute of Anatomy, always starting classes through a visit to the Anatomy Museum;
- ✓ practical example on dissection specimens, atlases;
- ✓ continuous adaptation of the content of practical works to current requirements and trends in the field of anatomy;
- ✓ making multidisciplinary links (medical imaging, laboratory medicine, etc.) that help to better understand the functionality of the structures studied and resumed by other disciplines.

Didactic position gained through contest:

- Lecturer – since February 2016 until present, Department of MorphofunctionalSciences I , Chair of Anatomy, Faculty of Medicine, ”Grigore T. Popa” University of Medicine and Pharmacy Iași.
- Assistant professor – since October 2008 until February 2016, Department ofMorphofunctionalSciences I, Chair of Anatomy, Faculty of Medicine, ”Grigore T. Popa” University of Medicine and Pharmacy Iași.
- Associate instructor – since march 2005 until October 2008, Chair of Anatomy, Faculty of Medicine, ”Grigore T. Popa” University of Medicine and Pharmacy Iași.

Between 2002 and 2018, I taught both courses and laboratory hours at the Department of Anatomy and Embryology at all study directions - Medicine, Dentistry and Pharmacy. I have taught students of Medicine and Dentistry faculties notions of course and practical laboratories in both romanian and english study programs.

To complete the psycho-pedagogical abilities and the specific methodical skills for superior education, I followed a post-university internship course for General Psychopedagogy organized by ”Al. I. Cuza” University, with graduation certificate seria F, no. 0022142.

In 2010, after several specific exams, I gained the certificate for English language abilities (B2 level), approved by the Ministry of Education, Research and Youth and also by the Ministry of Work, Social Solidarity and Family(Certificate 45/09.06.2010 – Series B, no. 0048595).

Promoting to the title of Lecturer at the same Department I have been teaching the courses of Anatomy and Embryology to the Ist and 2nd academic year, for both English and Romanian series of students. I have focused the courses on student involvement and at the same time on developing the capacity for expression and exposure in an academic way.

I coordinated a number of 4 graduation papers, of which 3 are personal research and one is a review. At the same time, I directed a number of 12 students who held 5 oral presentations and 2 posters at Students Congressis and Congress of the Society of Anatomists in Romania.

I wrote, as member in a group of authors, four books in anatomy field.

I was an invited lecturer at four workshops organized by the Faculty of Theater, ”George Enescu” University of Arts in Iaşi, represented by Associate Professor Dr. Irina Andreea Scutariu.

1.3. **Scientific activities**

I have begun my scientific research activity at the same time with the enrollment in the PhD studies. I have been admitted to be PhD training at the ‘Grigore T. Popa’ University of Medicine and Pharmacy Iaşi in 2008 and in 2011 I obtained the title of Doctor in Medical Sciences (O.M. 6468, from december 2011) – with the thesis entitled: ”Macro and microscopic vascularisation of the rectum and implications in the diagnosis and prognostics of colorectal carcinomas”, scientifically coordinated by Prof. Doina Lucia Frîncu. During the preparation of the PhD thesis I updated and improved series of anatomical dissection techniques of pelvic structures.

I chose this theme due to the relatively high incidence of affection in the Moldavian area, being 15.23 per 100,000 inhabitants in Iasi County between 2004 and 2007, but also due to the unsatisfactory efficiency of the early diagnosis of colorectal cancer.

The main objective of the thesis was to propose a diagnostic screening algorithm for early detection of colorectal cancer as a result of systematization of anatomoclinical and anatomopathological criteria in early diagnosis of colorectal carcinomas, evaluation of risk factors and their involvement in the prognosis of the disease. The methods used for this study

have yielded reproducible results in both laboratory and current medical and didactic practice.

The macroscopic evaluation of the surgery specimens is decisive for the determination of postoperative risks and complications. At the same time, their microscopic, quantitative and qualitative assessment is determinant for prognosis and subsequent treatment.

I actively participated in the colon and rectal surgey operations for the neoplasms located at this level and all the analyzed specimens are from these patients.

Another challenge I had was familiarity with common coloring techniques (hematoxylinosine, etc.) and microscopic examination, with the interpretation of the results, under the guidance of the scientific PhD coordinator as well as with the immunohistochemistry.

The anatomical study, another strength of the thesis, is due to the extraordinary opportunity for my training in dissection and the study of the pelvic-perineal structures with the operative microscope (Kaps), as well as the mesoscopic imaging.

Quantitative measurements were made in the Quantitative Microanatomy Laboratory of the "Ion Iancu" Institute of Anatomy in "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, on representative microscopic sections with a 40x lens. The images were taken from the microscope using an image acquisition system (PC camcorder), followed by the PRODIT 5.2 professional program.

I want to highlight the importance of the anatomoimagistical study that we have reached up to the 3D pelvic reconstruction. I started from the colonoscopy and barium enema techniques and I reached to interpret hundreds of CT and MRI images with or without contrast substance and in various incidences which were extremely useful in later studies on other organs.

The objectives proposed and achieved through the doctoral research project have a strong practical and scientific impact. The practical impact is primarily on population health, awareness of the risk factors involved in the processes of colorectal tumor development and the possibilities of early detection. The study also has strong implications in medical imaging or oncology, bringing the anatomical evidence underlying and capable of underpinning future early diagnosis and treatment techniques and protocols.

The theme leaves open other gates for research into the neoangiogenesis mechanisms involved in the development of colorectal tumors. Quantitation of tumor angiogenesis may allow patients to be divided by the type of treatment and may allow for prospective management selection of individuals with low microvascular tumor density.

My postdoctoral evolution developed towards: scientific direction, meaning research, professional direction, meaning medical practice and academic direction, meaning universityteaching. These levels are strongly interconnected, the teaching and medical practice being in relationship with the research part.

Valorisation of the doctoral study results was achieved by:

- participation in 31 national conferences / symposiums and 7 international congresses with scientific papers;
- publication of scientific papers in ISI journals - 29 articles or BDI journals- 29 articles

- two of my articles were rewarded by UEFISCDI (Executive Agency for Higher Education, Research, Development and Innovation Funding):
    - Hînganu D, Scutariu MM, Hînganu MV. The existence of labial SMAS — Anatomical, imaging and histological study. ANNALS OF ANATOMY-ANATOMISCHER ANZEIGER, 2018, 218: 271-275. https://doi.org/10.1016/j.aanat.2018.04.009
    - Hînganu MV, **Hînganu D**, Cozma SR, Asimionoaiei-Simionescu C, Scutariu IA, Ionesie DS, Haba D. Morphofunctional evaluation of buccopharyngeal space using three-dimensional cone-beam computed tomography (3D-CBCT). Annals of Anatomy, 2018, 220: 1–8.

My capacity to organize and manage teaching is demonstrated by the published books, by the activity of coordinating diploma thesis and papers presented by students to scientific national and/or international congresses.

I wrote four anatomy books for students for study in Romanian, English and Franch.

INTERNATIONAL VISIBILITY is reflected by:

- Web of Science H-index: 7;
- total number of citations: 85;
- ISI papers 24 (of which 16 main author);
- articles BDI: 29 (of which 20 main author);
- participation in experience exchanges with other centers through the Erasmus + Mobility of Teachers Program, from abroad.
- Using the ORCID platform that provides a persistent identity for humans

1.4. **Research projects – future plans**

1.4.1. Researching on the existence of a morpho-functional relationship between the anterior parathyroid glands and the thymus

For centuries, the thymus was considered an enigmatic organ with unknown functions.

The discovery of the crucial role that thymus plays in the good functioning of the immune system occurred in 1961, when it turned out that laboratory mice that were thymectomised immediately after birth had a poor development of lymphoid tissue, decreased immune responses, and increased susceptibility of recurrent infections.

The development connection between the thymus and parathyroid organs during early organogenesis led us to the hypothesis that these organs may have overlapping functions.

However the primary functions of the thymus and parathyroid glands are quite distinct, with the thymus playing a critical role in producing T cells and parathyroid controlling calcium physiology through the production of PTH.

In terms of parathyroid involvement with the thymus in early organogenesis, prior to the separation of the two organs, the parathyroid domain expresses Ccl21, a chemokine that contributes to help recruit lymphoid cells to the thymus.

Our main interest in the research of this difficult domain is to demonstrate the existence or the default of a functional interrelation between thymus and parathyroid glands.

1.4.2. The fineness of hearing

This project will be developed in parallel with the study of the morpho-functional mechanisms underlying the singing voice.

The working method will be 3D-CBCT through which we can acquire linear and volumetric data about the eardrum house and the inner ear of canto artists. This allows 3D reconstruction and printing techniques to be used to identify the morphological and, implicitly functional, acquired or inborn features.

I will use a group of subjects without specific vocal training. Subjects are required to emit a vowel from the extremities of the vocal ambitus and then mimic the phonation.

Thus, we will be able to detect the changes occurring within the middle and internal ear in these subjects compared to the control group.

I intend to begin these projects in an immediate future and to apply for funding by submitting to national and international grant projects for this purpose.

1.4.3. The anticipated impact of implementing new research directions on the academic career and personal international visibility

Funding and running these projects allows us to achieve results with a strong practical impact. Thus, the results obtained will allow improvement of the canto training techniques by focusing on the role of the most active structures in the determinism of vocal abilities. They will implicitly enable the faster and more accurate realization of appropriate motor patterns by guiding canto techniques to direct stimulation of the reflex centers involved.

The results are also directly applicable in teaching activity of canto techniques for pupils and students who wish to pursue a career by educating the artistic voice in any field.

The applicability in the field of otorhinolaryngology is evident especially in phoniatrics and audiology, through the targeted orientation of the prophylaxis techniques of the diseases of phonatory apparatus on the anatomic structures with high risk.

Funding the dissemination of results by publishing articles in ISI rated journals with a high impact factor will allow my international visibility to grow as a researcher in the field.

1.4.4. Management and future prospectives

1.4.4.1. *Directions and principles for further research in SMAS*

I intend to perform this research in cooperation with Moebius Syndrome Foundation, MFOMS and other international health care and study groups related to oculofacial atresia. In order to this I propose a project which I have already started to work on, entitled ”*Aging face anatomical and embryological background”* and combines the concepts of SMAS with congenital nerve facial atresia.

The project will propose the evaluation and centralization of patients with Moebius syndrome and possible new cases from the North-East region of Romania. After their clinical evaluation, they will be classified according to the type of facial nerve damage, which is a criterion for establishing a clinical and paraclinical assessment protocol for them.

We will relate our findings to international currently knowledge.

All of our clinical work will be related to the genetic study we want to perform. We want to learn more about the genetics and clinical characteristics of Moebius syndrome and to explore the genetic and/or environmental causes of this condition.

This is a natural history study with a cross-sectional design of a heterogeneous developmental disorder defined as a congenital, non-progressive facial weakness with limited abduction of one or both eyes, often associated with additional features such as other cranial nerve dysfunction, craniofacial, skeletal and limb deformities, as well as intellectual or behavioral impairments.

In the study we will attempt to characterize the clinical phenotype of Moebius and associated congenital facial weakness syndromes, collect thorough information on possible prenatal environmental exposures and use genetic studies, including whole exome sequencing, on DNA from patients and family members of patients to identify disease-causing genes.

We will conduct brain MRI, fMRI and diffusion tensor imaging, in order to explore brainstem and cranial nerve structure and associated white matter tract anomalies.

Through this combined clinical, molecular and imaging approach, we anticipate that phenotype-genotype correlations will be revealed. These results will lead to new insights into the clinical definition of these conditions, molecular pathways, and potential networks involved in the pathogenesis of facial weakness and associated multisystem dysmorphogenesis.

1.4.4.2. *Incentives, directions and principles for the continuity of the research on singing voice.*

I will carry out this research mainly by using new exploration techniques such as fMRI *to mark and trace the centripetal pathways of the sensations triggered by the sound stimulus sung at the laryngeal, hypopharyngeal and oral cavity.*In order to thisI propose to perform mucosal and vocal muscles experiments to detect types and subtypes of nerve receptors at that level.

Meanwhile I intend to keep myself updated constantly in the field of neurolaryngology by participating to the main congresses and conferences worldwide.

The term *neurolaryngology* has come into use over the last 20 years or so.Neurolaryngology arose from collaborations between laryngologists and neurologists who recognized that neurologic disorders could impair laryngeal function, and that neurogenic etiology could be discerned frequently for seemingly idiopathic hoarseness and dysphagia.

The focus of neurolaryngology is the management of neurogenic dysfunction of the larynx and pharynx, including central and peripheral disorders. The focus is functional management, and thus surgical procedures are primarily rehabilitative and not extirpative.

My particular interest is to develop a new research area within neurolaryngology and phoniatrics, directly addressed to the singing voice.

Voice research and focal themes of interest have evolved acrosstimes. The demands of professional voice use currently play animportant role in clinical research and singing is no exception. These people depend on their vocal abilities at a high level of proficiency demands. Among them, singers are considered the elite of the vocal performers.

Such an activity requires more endurance, flexibility and vocal tract control.The combination of text mining andbibliometric techniques allows the identification of unseen patterns in research fields. Bibliometrics and scientometrics arerelatively unexplored from both qualitative and quantitativeperspectives.

Thus, this future research in the fieldcompulsory needs to shed new light on the identification of major academic branches and research trends in singing voice.

1.4.4.3. *Further research of neovasculogenesis processes in colorectal and cervical cancers - perspectives and anticipated approach*

The main approach of this field consists in parallel studies of the paraneoplastic neovasculogenesis phenomena that occur in the most common cancers with pelvic and perineal localization. I shall do this by using nowadays more advanced radiologic techniques, such as angioMRI, angioCT and PET-CT techniques.

Thin section T2-weighted magnetic resonance imaging (MRI) can be used to demonstrate a number of prognostic features for local disease recurrence and survival in patients with pelvic cancers, which were initially established from histopathological studies. These include tumour extension to the lateral resection margin, the depth of extramural tumour extension and the presence of extramural venous invasion (EMVI).

The regular outer wall of the pelvic organsare perforated by numerous small venules, which appear as low to intermediate signal intensity tubular structures at T2-weighted MR imaging. Extramural venous invasion is recognised at T2-weighted MR imaging by the expansion and irregularity of these venules adjacent to the primary tumour, due to contiguous tumour extension.

The involved vein usually appears intermediate signal intensity with loss of the normal vascular flow void.

Lymphatic drainagesystem consists of channels smaller than blood vessels and can notbe directly visualised at T2-weighted MRI. We consider that EMVI scoring may act as a surrogate marker for lymphatic permeation by tumour.

Patients with MR evidence of extramural venous invasion will carry a higher risk and incidence of nodal disease. However, the potential relationship between MRI determined extramural venous invasion and the presence of nodal disease has not been previously investigated.

The recognition of MRI imaging features that are associated with increased risk of nodal disease can alert the radiologist to carefully perform the staging examination for nodal disease. An association between EMVI and nodal disease, particularly stage N2 nodal involvement, can also strengthen the argument for neoadjuvant chemoradiation in patients demonstrating such features, with the aim of reducing local disease recurrence.

Even if the EMVI scoring system have been applied just on rectal cancer until now, our clinical experience shows it could be an useful tool in staging another pelvic cancers, such as cervical, endometrial and vesicale.

SECTION III

References

1. Abd-EL-Malek S. A contribution to the study of the movements of the tongue in animals, with special reference to the cat. J Anat 1938; 73:15–30.
2. Adam JA. A mathematical model of tumor growth II: effects of geometry and spatial nonuniformity on stability. Math Biosci 1997; 86:183-211.
3. Akagi K, Ikeda Y, Sumiyoshi Y, Kimura Y, Kinoshita J, Miyazaki M, Abe T. Estimation of angiogenesis with anti-CD105 immunostaining in the process of colorectal cancer development. Surgery 2002; 131(1): 109-113.
4. Al-Ekrish A, Ekram M. A comparative study of the accuracy and reliability of multidetector computed tomography and cone beam computed tomography in the assessment of dental implant site dimensions. Dentomaxillofac Radiol 2011; 40:67-75.
5. Al-Sukhni E, Messenger DE, Charles Vicor J, McLeod RS, Kennedy ED. Do MRI reports contain adequate preoperative staging information for end users to make appropriate treatment decisions for rectal cancer? Ann Surg Oncol 2013; 20:1148-55.
6. Alberda WJ, Verhoef C, Nuyttens JJ et al. Outcome in patients with resectable locally recurrent rectal cancer after total mesorectal excision with and without previous neoadjuvant radiotherapy for the primary rectal tumor. Ann Surg Oncol 2014; 21(2): 520-526.
7. Alberda WJ, Verhoef C, Nuyttens JJ, van Meerten E, Rothbarth J, de Wilt JH et al. Intraoperative radiation therapy reduces local recurrence rates in pa- tients with microscopically involved circumferential resection margins after resection of locally advanced rectal cancer. Int J Radiat Oncol Biol Phys 2014; 88(5): 1032e40.
8. Allison JE. Colorectal cancer screening guidelines: the importance of evidence and transparency. Gastroenterology 2010; 138:1648.e2–1652.e2.
9. Almeida SI. 105 years of phoniatry. Braz J Otorhinolaryngol 2010; 76(3): 275.
10. American Cancer Society: Special section: Colon & rectum cancer. In: Cancer facts & figures - 1999; Atlanta: 1999;18-23.
11. Arai Y, Tammisalo E, Iwai K, Hashimoto K, Shinoda K. Development of a compact computed tomographic apparatus for dental use. Dentomaxillofac Radiol 1999; 28:245-248.
12. Assaneo MF, Sitt J, Varoquaux G, Sigman M, Cohen L, Trevisan MA. Exploring the anatomical encoding of voice witha mathematical model of the vocal system. Neuroimage 2016; 141:31–39.

13. Athanasiou A, Rempelos G. Lore's Fascia a Strong Fixation Point for Neck Rejuvenation Procedures. Clinics in Plastic Surgery 2014; 41(1): 43-49.
14. Atkin WS, Edwards R, Wardle J et al. Design of a multicentre randomized trial to evaluate flexible sigmoidoscopy in colorectal cancer screening. J Med Screen 2001; 8(3): 137–44.
15. Ayuso CJ, Pages LM, Ayuso CC. Estadification del cancer de recto. Radiologia 2010; 52:18-29.
16. Baek SH, Cho IS, Chang YI, Kim MJ. Skeletodental factors affecting chin point deviation in female patients with class III malocclusion and facial asymmetry: a three-dimensional analysis using computed tomography. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104(5): 628–639.
17. Bai-lin Pan. Upper lip lift with a “T”-shaped resection of the orbicularis oris muscle for Asian perioral rejuvenation: A report of 84 patients, Journal of Plastic, Reconstructive & Aesthetic SurgeryVolume 2017; 70:392–400.
18. Baish JW, Jain RK. Fractals and cancer. Cancer Res 2000; 60:3683-3688.
19. Bakkar AA, Wallerand H, Radvanyi F, Lahaye JB, Pissard S, Lecerf L, Kouyoumdjian JC, Abbou CC, Pairon JC, Jaurand MC, Thiery JP, Chopin DK, de Medina SG. FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder. Cancer Res 2003; 63:8108-8112.
20. Barlow SM, Stumm S. Speech Production: Adults.In: Squire L.R. (Ed.) Encyclopedia of Neuroscience, Academic Press, Oxford 2009; pp. 247-254.
21. Barton FE. The SMAS and the nasolabial fold. Plastic and Reconstructive Surgery1992; 89:1054-1059.
22. Beets-Tan RG, Beets GL, Borstlap AC et al. Preoperative assessment of local tumor extent in advanced rectal cancer: CT or high-resolution MRI? Abdom Imaging 2000; 25:533-41.
23. Beets-Tan RG, Lambregts DM, Maas M, et al. Magnetic resonance imaging for theclinical management of rectal cancer patients: recommendations from the 2012 European Society of Gastrointestinal and Abdominal Radiology (ESGAR) consen- sus meeting. Eur Radiol 2013; 23(9): 2522–2531.
24. Bergman RA, Thompson SA, Afifi A, Saadeh FA. Compendium of Human Anatomic Variation: Text, Atlas, and World Literature, Urban and Schwarzenberg, Munich, Germany, 1988.
25. Berke GS, Long JL. Functions of the larynx and production of sounds. In:Handbook of Behavioral Neuroscience 2009; 19:419-426.
26. Berry MG, Davies D. Platysma-SMAS plication facelift. J Plast Reconstr Aesthet Surg 2010; 63(5):793–800.
27. Bhangu A, Mohammed AS, Brown G, Nicholls RJ, Tekkis P. Indications and outcome of pelvic exenteration for locally advanced primary and recurrent rectal cancer. Ann Surg 2014; 259(2): 315-322.
28. Bhatla N, Aoki D, Sharma DN, Sankaranaraayanan R. Cancer of the cervix uteri. Int J Gyneacol Obstet 2018; 143(2).

29. Bird TG, Ngan SY, Chu J, Kroon R, Lynch AC, Heriot AG. Outcomes and prognostic factors of multimodality treatment for locally recurrent rectal cancer with curative intent. Int J Colorectal Dis 2018; 33(4): 393-401.
30. Blomqvist L, Rubio C, Holm T et al. Rectal adenocarcinoma: assessment of tumour involvement of the lateral resection margin by MRI of resected specimen. Br J Radiol 1999; 72:18-23.
31. Boisaubin EV. Cardiology in Ancient Egypt. Tex Heart Inst J 1988; 15:80–85.
32. Bokkerink GM, Buijs EF, de Ruijter W, Rosman C, Sietses C, Strobel R, et al. Improved quality of care for patients undergoing an abdominoperineal exci-sion for rectal cancer. Eur J Surg Oncol 2015; 41(2):201e7.
33. Bonanno PC, Casson PR. Frey's syndrome: a preventable phenomenon. Plast Reconstr Surg 1992; 89(3):452–456; discussion 457–458.
34. Bonjer HJ, Deijen CL, Abis GA, et al. A randomized trial of laparoscopic versus open surgery for rectal cancer. N Engl J Med 2015; 372:1324–1332.
35. Bono AV, Celato N, Cova V, Salvadore M, Chinetti S, Novario R. Microvessel density in prostate carcinoma. Prostate Cancer Prostatic Dis 2002; 5:123-127.
36. Bosari S, Lee AKC, DeLellis RA, Wiley B, Heatley GJ, Silverman ML. Microvessel quantitation and prognosis in invasive breast carcinoma. Hum Pathol 1992; 23:755-761.
37. Bosman SJ, Holman FA, Nieuwenhuijzen GAP, Martijn H, Creemers GJ, Rutten HJT. Feasibility of reirradiation in the treatment of locally recurrent rectal cancer. Br J Surg 2014; 101(10):1280-1289. doi:10.1002/bjs.9569.
38. Bosse JP, Papillon J. Surgical anatomy of the SMAS at the malar region. Transactions of the 9th International Congress of Plastic & Reconstructive Surgery 1987; 348-9.
39. Bourgioti C, Chatoupis K, Moulopoulos LA. Current imaging strategies for the evaluation of uterine cervical cancer. World J Radiol 2016; 8:342–354.
40. Bourgioti C, Chatoupis K, Rodolakis A, Antoniou A, Tzavara C, Koutoulidis V, Moulopoulos LA. Incremental prognostic value of MRI in the staging of early cervical cancer: a prospective study and review of the literature. Clin Imag 2016; 40(1):72-78.
41. Brenner H, Arndt V, Sturmer T, Stegmaier C, Zieger H, Dhom G. Long-lasting reduction of risk of colorectal cancer following screening endoscopy. Br J Cancer 2001; 85(7):972-6.
42. Brenner H, Chang-Claude J, Seiler CM, et al. Potential for colorectal cancer prevention of sigmoidoscopy versus colonoscopy: population-based case control study. Cancer Epidemiol Biomarkers Prev 2007; 16(3):494-499.
43. Briche D, Malka G, Chassagne JF. Vallécules: réalitéanatomique? Rev Stomatol Chir Maxillofac 1991; 92:213-218.
44. Brouwer NPM, Bos A, Lemmens V, Tanis PJ, Hugen N, Nagtegaal ID et al. Anoverview of 25 years of incidence, treatment and outcome of colorectal cancer patients. Int J Canc 2018; 143(11):2758e66.
45. Bryan CE. The Papyrus Ebers, Translated from the GermanVersion. London: Geoffrey Bles. 1930, p 124–132.
46. Bryce TH. Myology, in Quain's Elements of Anatomy, E. Sharpey, J. Symimgton, and T. H. Bryce, Eds, p. 32, Longman Green & Co, London, UK, 11th edition, 1923.

47. Burt, Randall W. Colorectal Cancer Screening. Curr Opin Gastroenterol 2010; 26(5):466-470.
48. Calnan J. Modern views on Passavant's ridge. British Journal of Plastic Surgery 1957-1958; 10:89-113.https://doi.org/10.1016/S0007-1226(57)80018-6.
49. Cantor SB, Càrdenas-Turanzas M, COX DD, Atkinson EN, Nogueras-Gonzalez GM, Beck JR, Follen M, Benedet JL. Accuracy of colposcopy in the diagnostic setting compared with the screening setting. Obstet Gynecol 2008; 111:7.
50. Cárdenas-Camarena L, González LE. Multiple, combined plications of the SMAS-platysma complex: breaking down the face-aging vectors. Plast Reconst Surg 1999; 104 (4): 1093–1100.
51. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011; 473: 298–307.
52. Carmichael JA, Jeffrey JF, Steele HD et al. The cytologic hystory of 245 patients developing invasive cervical carcinoma. Am J Obstet Gynecol 1984; 148: 685-690.
53. Cervantes A, Rodriguez-Braun E, Navarro S, Hernandez AS, Campos S, Garcia-Granero E. Integrative decisions in rectal cancer. Ann Oncol 2007; 18:127-31.
54. Chan O, Adams E, Clark C, Dabbs M, Jamieson C, Hussein M, Laing R, Langley S, Nisbet A, Guerrero Urbano T. Impact of Intrafraction Motion on TCP and Rectal NTCP Values in Patients Receiving IG-IMRT for Prostate Cancer. IntJ Radiat Oncol Biol Phys 2010; 78:363-364.
55. Chang F, Syrjanen S, Kurvinen K, Syrjanen K. The p53 tumor suppressor gene as a common cellular target in human carcinogenesis. Am J Gastroenterol 1993; 88(2):174-86.
56. Cidon EU, Alonso P, Masters B. Markers of Response to Antiangiogenic Therapies in Colorectal Cancer: Where Are We Now and What Should Be Next? Clin Med Insights Oncol 2016; 10(1):41-55.
57. Claassen H, Schicht M, Sel S, Werner J, Paulsen F.The fate of chondrocytes during ageing of human thyroid cartilage. Histochem Cell Biol 2009; 131:605-14.
58. Clément P, Hans S, Hartl DM, Maeda S,Vaissière J, Brasnu D. Vocal Tract Area Function for Vowels Using Three-Dimensional Magnetic Resonance Imaging. A Preliminary Study. J Voice 2007; 21:522-530.
59. Corines MJ, Nougaret S, Weiser MR, Khan M, Gollub MJ. Gadolinium-based con- trast agent during pelvic MRI: contribution to patient management in rectal can- cer. Dis Colon Rectum 2018; 61(2):193–201.
60. Costello P, McCann A, Carney DN, Dervan PA. Prognostic significance of microvessel density in lymph node negative breast carcinom. Hum Pathol 1995; 26(11):1181-1184.
61. Da Silva BB, Pires CG, dos Santos AR, de Castro-Leão AH, Alencar AP, Lopes-Costa PV. Effects of raloxifene on Ki-67 and CD34 antigen expression in breast cancer. Gynecol Obstet Invest 2009; 67(2):103-108.
62. Dankbaar JW, Pameijer FA. Vocal cord paralysis: anatomy, imaging and pathology. Insights Imaging 2014; 5(6):743–751.
63. de Castro CC. Superficial musculoaponeurotic system-platysma: a continuous study. Anna Plast Surg 1991; 26(3): 203–211.

64. De Cock J, Mermuys K, Goubau J, Van Petegem S, Houthoofd B, Casselman JW. Cone-beam computed tomography: a new low dose, high resolution imaging technique of t he wrist, presentation of three cases with technique. Skeletal Radiol 2012; 41(1):93-6.
65. De la Taille A, Kratz AE, Bagiella E, Buttvan R, Shair S, Olsson CA, Burchardt, Ennis RD, Rubin MA. Microvessel density as a predictor of PSA recurrence after radical prostatectomy. A comparison of CD34 and CD31. Am J Clin Pathol 2000; 113: 555-562.
66. Dell'Aversana Orabona G, Salzano G, Abbate V, Piombino P, Astarita F, Iaconetta G, Califano L. Use of the SMAS flap for reconstruction of the parotid lodge. Acta Otorhinolaryngol Ital 2015; 35(6):406–411.
67. Delmar H. Anatomie des plans superficiels de la face et de cou. Ann Chirurg Plast Esthet 1994; (39)5:527-55.
68. Double AF. Traité Des Variations Du Systéme Musculare De L'Homme. Paris: Schleicher; 1897.
69. Du Z, Fujiyama C, Chen Y, Masaki Z. Expression of hypoxia-inducible factor 1alpha in human normal, benign, and malignant prostate tissue. Chin Med J (Engl) 2003; 116: 1936-1939.
70. Duchenne de Boulogne GB. The Mechanism of Human Facial Expression. Ed. Cuthbertson A. Cambridge University Press. Hueston JT (1990) 3. Duchenne today: Facial expression and facial surgery.
71. Duska L. Recognising endometrial cancer in premenopausal women. BJOG 2017; 124:412-412.
72. Ebbel B. The Papyrus Ebers: The Greatest Egyptian Medical Document. Copenhagen: Levin and Munksgaard. 1937; p 114–117.
73. Edwards BK, Ward E, Kohler BA, et al. Annual report to the nation on the status of cancer 1975–2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer 2010; 116:544–73.
74. Fabre Ph: Un procédé électrique percutané d'inscription de l'accolement glottique ou cours de la phonation: glottographie de haute fréquence; premiere résultats. Bull Acad Nat Med 1957: 66-69.
75. Fedok FG, Chaikhoutdinov I, Garritano F. The difficult neck in facelifting. Facial Plast Surg 2014; 30(4):438-450.
76. Fehrenbach MJ. Anatomiailustrada da cabeça e dopescoço. Ed. Manole, 1998; ISBN-13: 9788520407417.
77. Ferlay J, Bray F, Pisani P, Parkin DM: GLOBOCAN 2002: Cancer incidence, mortality and prevalence worldwide. International Agency for Research on Cancer Press, Lyon, France, 2004, 5, Version 2.0.
78. Fleshman J, Branda M, Sargent DJ, et al. Effect of Laparoscopic-Assisted Resection vs Open Resection of Stage II or III Rectal Cancer on Pathologic Outcomes. JAMA 2015; 314(13):1346. doi:10.1001/jama.2015.10529.
79. Fleshman J, Branda ME, Sargent DJ et al. Disease-free survival and local recurrence for laparoscopic resection compared with open resection of stage II to III rectal cancer: follow-up results of the ACOSOG Z6051 randomized controlled trial. Ann Surg Epub ahead of print 2018.

80. Fogli A. Rajeunissement jugal et traitement des bajoues Jugal rejuvenation and jowls treatment. Annales deChirurgie Plastique Esthétique, 2017; 62: 424-434.
81. Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol 1992; 3(2):65–71.
82. Folkman J. Tumor angiogenesis: Therapeutic implications. N Engl J Med 1971; 285(21):1182–1186.
83. Francu LL, Hinganu D, Hinganu MV. Anatomical evidence regarding sustentaculum facies. Rom J Morphol Embryol 2013; 54(3 Suppl):757–761.
84. Fujita S, Mizusawa J, Kanemitsu Y et al. Mesorectal excision with or without lateral lymph node dissection for clinical stage II/III lower rectal cancer (JCOG0212): a multicenter, randomized controlled, noninferiority trial. Ann Surg 2017; 266:201–207.
85. Gardetto A, Dabernig J, Rainer C, Piegger J, Piza-Katzer H, Fritsch H. Does a superficial musculoaponeurotic system exist in the face and neck? An anatomical study by the tissue plastination technique. Plast Reconstr Surg 2003; 111(2):664-672.
86. Gasser RF. The development of the facial muscles in man. Am J Anat 2005; 120(2):357-375.
87. Gheorghe L, Gheorghe C, Cazacu M. Cancerul colorectal. In: Grigorescu M, editor. Tratat de Gastroenterologie. Bucureşti: Editura Medicala Naţională; 2001; 139-73.
88. Giampapa VC, Di Bernardo BE. Neck recontouring with suture suspension and liposuction: an alternative for the early rhytidectomy candidate. Aesthetic Plast Surg 1995; 19(3): 217-223.
89. Giannone N, Lo Muzio L, Politi M. Extracapsular lumpectomy and SMAS flap for benign parotid tumours: an early outcome in a small number of cases on Frey's syndrome and facial nerve dysfunction. J Craniomaxillofac Surg 2008; 36(4):239–243.
90. Goddard JC, Sutton CD, Furness PN, O'Byrne KJ, Kockelbergh RC. Microvessel density at presentation predicts subsequent muscle invasion in superficial bladder cancer. Clin Cancer Res 2003; 9: 2583-2586.
91. Goerttler K: Die Anordnung, Histologie und Histogenese der quergestreiften Muskulatur im menschlichen Stimmband. Zeitschrift für Anatomie und Entwicklungsgeschichte, Ludwigs-Universitat Freiburg 1951: 352-401.
92. Goh KSK, Ong TA, Peh SC, Yuen HL, Naicker M. P53 Expression in Colorectal Carcinoma: The University of Malaya Medical Centre's Experience. Med J Malaysia 2004; 59(4):515-21.
93. Goh V, Padhani AR, Rasheed S, Functional imaging of colorectal cancer angiogenesis. Lancet Oncol 2007; 8(3):245-255.
94. Gola R, Chossegros C, Carreau P. [Surgical anatomy of the parotid region. Current concepts]. Rev Stomatol Chir Maxillofac 1994; 95(6):395–410.
95. Gola R. Chirurgie esthétique et fonctionnelle de la face. Springer-Verlag, Paris, France, 2005.
96. Goldis DS, Sferdian MF, Tarţă C, Fulger LO, Totolici BD, Neamţu C. Comparative analysis of microvessel density quantified through the immunohistochemistry expression of CD34 and CD105 in rectal cancer. Rom J Morphol Embryol 2015; 56(2):419.

97. Gollub MJ, Arya S, Beets-Tan RG et al. Use of magnetic resonance imaging in rectal cancer patients: society of Abdominal Radiology (SAR) rectal cancer disease-focused panel (DFP) recommendations 2017. Abdom Radiol (NY) 2018; 43(11):2893–2902.
98. Gollub MJ, Lakhman Y, McGinty K, et al. Does gadolinium-based contrast material improve diagnostic accuracy of local invasion in rectal cancer MRI? A multireader study. Am J Roentgenol 2015; 204(2):160–167.
99. Gondal G, Grotmol T, Hofstad B, Bretthauer M, Eide TJ, Hoff G: The Norwegian Colorectal Cancer Prevention (NORCCAP) screening study. Scand J Gastroenterol 2003; 38(6):635–42.
100. Gosain AK, Klein MH, Madiedo G, Larson DL, Matloub HS, Sanger JR. Surgical anatomy of the SMAS: a reinvestigation. Plast Reconstr Surg 1993; 92(7):1254-63.
101. Greene FL, Page D, Morrow M. AJCC cancer staging manual, 6thed. New York, Springer 2002; 113-23.
102. Hall NR, Fish DE, Hunt N, Goldin RD, Guillou PJ, Monson JRT, Is the relationship between angiogenesis and metastasis in breast cancer real? Surg Oncol 1992; 1:223-229.
103. Hansen TF, Andersen CL, Nielsen BS, Spindler KGS, Sorensen FB, Lindebjerg J, Brandslung I, Jakobsen A. Elevated microRNA-126 is associated with high vascular endothelial growth factor receptor 2 expression levels and high microvessel density in colorectal cancer. Oncology Letters 2011; 2(6): 1101–1106.
104. Harris CA, Solomon MJ, Heriot AG et al. The Outcomes and Patterns of Treatment Failure After Surgery for Locally Recurrent Rectal Cancer. Ann Surg 2016; 264(2):323-329. doi:10.1097/SLA.0000000000001524.
105. Harris CRS. The Heart and the Vascular System in Ancient Greek Medicine. Oxford: Oxford University Press 1993; p 1–28.
106. Harvey W. On the Motion of the Heart and Blood in Animals. 1962; Chicago: H. Regnery Co.
107. Hecking T, Abramian A, Domro€se C et al. Individual management of cervical cancer in pregnancy. Arch Gynecol Obstet 2016; 293:931–939.
108. Hein E, Rogalla P, Klingebiel R, Hamm B. Low-dose CT of the paranasal sinuses with eye lens protection: effect on image quality and radiation dose. Eur Radiol 2002; 12:1693–1696.
109. Henry ZH, Yeaton P, Shami VM et al. Gastrointest Endosc 2010; 72(1):118.
110. Herbst C, Kosmehl H, Stiller KJ, Berndt A, Eiselt M, Schubert J. Evaluation of microvessel density by computerized image analysis in human renal cell carcinoma. Correlation to pT category, nuclear grade, proliferative activity and occurrence of metastasis. J Cancer Res Clin Oncol 1998; 124:141-147.
111. Herbst CT. A review of singing voice subsystem interactions – toward an extended physiological model of "support". J Voice 2017; 31(2): 249.e13–249.e19.
112. Heriot AG, Hicks RJ, Drummond EG et al. Does positron emission tomography change management in primary rectal cancer? A prospective assessment. Dis Colon Rectum 2004; 47: 451-458.
113. Hewett DG, Kahi CJ, Rex DK. Does colonoscopy work? J Natl Compr Canc Netw 2010; 8:67–76.

114. Hewitson P, Glasziou P, Irwig L, Towler B, Watson E: Screening for colorectal cancer using the faecal occult blood test, Hemoccult. Cochrane Database Syst Rev 2007; 1, CD001216.
115. Heymann O. Die Stroboskopie im Dienst der Laryngoskopie. Archiv f Ohren-Nasen-Kehlkopfheilk 1933; 136: 64-116.
116. Hiramatsu H, Tokashiki R, Nakamura H, Motohashi R, Sakurai E, Nomoto M, Toyomura F, Suzuki M. Analysis of High-Pitched Phonation Using Three-Dimensional Computed Tomography. J Voice 2012; 26:548-554
117. Holman FA, Bosman SJ, Haddock MG et al. Results of a pooled analysis of IOERT containing multimodality treatment for locally recurrent rectal cancer. Eur J Surg Oncol 2017; 43(1):107-117. doi:10.1016/j.ejso.2016.08.015.
118. Homan J, Bokkerink GM, Aarts MJ, Lemmens VE, van Lijnschoten G, Rutten HJ et al. Variation in circumferential resection margin: reporting and involve- ment in the South-Netherlands. Eur J Surg Oncol 2015; 41(11):1485e92.
119. Horn LC, Fischer U, Raptis G et al. Tumor size is of prognostice value in surgically treated FIGO syage II cervical cancer. Gynecol Oncol 2007; 107:310-315.
120. Hricak H, Powell CB, Yuk K et al. Invasive cervical carcinoma: role of MR imaging in pretreatment work-up: Cost minimization and diagnostic efficacy analysis. Radiology 2014; 198: 403.
121. Hundt S, Haug U, Brenner H. Comparative evaluation of immunochemical fecal occult blood tests for colorectal adenoma detection. Ann Intern Med 2009; 150:162-9.
122. Husson R: La Voix chantée : Commande cérébrale des cordes vocales, classification chronaximétrique des tessitures, mécanismes protecteurs du larynx, analyse physiologique des techniques vocales et des grandes méthodes pédagogiques. Gauthier-Villars Gap, impr. L. Jean, 1960: 40-65.
123. Husson R: Propriétés fondamentales de la neuro-musculature des cordes vocales au cours de la phonation. Journ. de Physiol 1951; 43: 757-8.
124. Husson R., 1960. La Voixchantée: Commandecérébrale des cordesvocales, classification chronaximétrique des tessitures, mécanismesprotecteurs du larynx, analyse.Villars Gap, impr. L. Jean, 40-65.
125. Ikoma R, Ishitoya J, Sakuma Y, Hirama M, Shiono O, Komatsu M, Oridate N. Temporary facial nerve dysfunction after parotidectomy correlates with tumor location. Auris Nasus Larynx 2014; 41(5):479–484.
126. Jankowski J, Sampliner R, Kerr D, Fong Y. Gastrointestinal Oncology: A Clinical Multidisciplinary Team Approach. Blackwell Publishing 2008.
127. Jayne D, Pigazzi A, Marshall H et al. Effect of robotic-assisted vs conventional laparoscopic surgery on risk of conversion to open laparotomy among patients under- going resection for rectal cancer: the ROLARR randomized clinical trial. JAMA 2017; 318:1569–1580.
128. Jeffrey C, Posnick DMD. Aesthetic Alteration of the Soft Tissues of the Neck and Lower Face: Evaluation and Surgery. Orthognathic Surgery Principles and Practice 2015; pp. 1746-1843.
129. Jemal A, Thomas A, Murray T, Thom M. Cancer statistics 2002. CA Cancer J Clin 2002; 52:9-25.

130. Jemal A, Tiwar RC, Murray T. Cancer statistics 2004. CA Cancer J Clin 2004; 54:8-29.
131. Jeong S-Y, Park JW, Nam BH et al. Open versus laparoscopic surgery for mid-rectal or low-rectal cancer after neoadjuvant chemoradiotherapy (COREAN trial): survival outcomes of an openlabel, noninferiority, randomised controlled trial. Lancet Oncol 2014; 15:767–774.
132. Johnson CD, Chen MH, Toledano AY et al. Accuracy of CT colonography for detection of large adenomas and cancers. N Engl J Med 2008; 359(12):1207-1217.
133. Jonker FHW, Hagemans JAW, Burger JWA, Verhoef C, Borstlap WAA, Tanis PJ et al. The influence of hospital volume on long-term oncological outcome after rectal cancer surgery. Int J Colorectal Dis 2017; 32(12):1741-7.
134. Jonker FHW, Hagemans JAW, Verhoef C, Burger JWA. The impact of hospital volume on perioperative outcomes of rectal cancer. Eur J Surg Oncol 2017; 43(10):1894e900.
135. Jose AM. Anatomy and Leonardo da Vinci. Yale J Biol Med 2001; 74:185–195.
136. Joshi MM, Joshi SS, Joshi SD. Morphometric study of thyroidcartilages in western India. Int J Anat Res 2015; 3(2):1028–1033.
137. Jost G, Levet Y. Parotid fascia and face lifting: a critical evaluation of the SMAS concept. Plast Reconstr Surg 1984; 74(1):42–51.
138. Kahi CJ, Imperiale TF, Juliar BE et al. Effect of screening colonoscopy on colorectal cancer incidence and mortality. Clin Gastroenterol Hepatol 2009; 7(7):770-775.
139. Kahlenberg M, Stoler D, Rodriguez-Bigas MA, Weber TK, Driscoll DL, Anderson GR, Petrelli NJ. P53 tumor suppressor gene mutations predict decreased survival of patients with sporadic colorectal carcinoma. Cancer 2000; 88(8): 1814-9.
140. Kahlenberg MS, Sullivan JM, Witmer DD, Petrelli NJ. Molecular prognostics in colorectal cancer. Surg Oncol 2003; 12(3): 173-86.
141. Karagkounis G, Stocchi L, Lavery IC, et al. Multidisciplinary Conference and Clinical Management of Rectal Cancer. J Am Coll Surg 2018; 226(5):874-880.
142. Katepratoom C, Manchana T, Amornwichet N. Lower urinary tract dysfunction and quality of life in cervical cancer survivors after concurrent chemoradiation versus radical hysterectomy. Int Urogynecol J 2014; 25:91–96.
143. Kaur R, Singla RK, Laxmi V. The morphology and morphometry of adult human thyroid cartilage – a cadaveric study in north Indian population. CIBTech J Surg 2014; 3(1):18–26.
144. Khan HA, Bagheri S. Management of the superficial musculoaponeurotic system (SMAS). Atlas Oral Maxillofac Surg Clin North Am 2014; 22(1):17–23.
145. Khemthongcharoen N, Jolivot R, Rattanavarin S, Piyawattanametha W. Advanced Drug Delivery Reviews 2014; 74(30):53.
146. Kishan AU, Voog JC, Wiseman J et al. Standard fractionation external beam radiotherapy with and without intraoperative radiotherapy for locally recurrent rectal cancer: the role of local therapy in patients with a high competing risk of death from distant disease. Br J Radiol 2017; 90(1076):20170134.

147. Kitamura T, Honda K, Takemoto H. Individual variation of the hypopharyngealcavities and its acoustic effects.Acoust. Sci. & Tech 2005; 26:16-26.
148. Klessen C, Rogalla, P, Taupitz M. Local staging of rectal cancer: the current role of MRI. Eur Radiol 2007; 17:379.
149. Kobayashi K, Shimoda S, Nakagawa Y, Yamamoto A. Accuracy in measurement of distance using limited cone-beam computerized tomography. Int J Oral Maxillofac Implants 2004; 19: 228-31.
150. Krasna MJ, Flancbaum L, Cody RP, Shneibaum S, Ben Ari G. Vascular and neural invasion in colorectal carcinoma. Incidence and prognostic significance. Cancer 1988; 61(5):1018-23.
151. Kurman RJ. Blaunstein's Pathology of the Female Genital Tract, Springer, 2002.
152. Kutta H, Steven P, Paulsen F. Anatomical definition of the subglottic region. Cells Tissues Organs 2006; 184:205-214.
153. Kutta H. Knipping S, Claassen H, Paulsen F. Update Larynx: funktionelle Anatomie unterklinischen Gesichtspunkten Teil I: Entwicklung, Kehlkopfskelett, Gelenke, Stimmlippenansatz, Muskulatur, HNO 2007, Springer MedizinVerlag 55: 583–598.
154. Laget P. Reproduction expérimentale de la vibration des cordes vocales, en l'absence de tout courant d'air, par stimulation électrique d'un récurrent de chien, avec observation stroboscopique de la réponse laryngée. Rev Laryng Portmann Suppl Février 1953; 132-142.
155. Le Louarn C, Buthiau D, Buis J. 2006. Rajeunissement facial et lifting malaire concentrique: le concept du face recurve. Annales de chirurgie plastique esthétique 2006; 51:99–121.
156. Lee CC, Chan RCL, Chan JYW. Predictors for Frey syndrome development after parotidectomy. Ann Plast Surg 2017; 79(1): 39–41.
157. Lee DJ-K, Sagar PM, Sadadcharam G, Tan K-Y. Advances in surgical management for locally recurrent rectal cancer: How far have we come? World J Gastroenterol 2017; 23(23):4170. doi:10.3748/wjg.v23.i23.4170.
158. Lee L, Burke JP, deBeche-Adams T et al. Transanal minimally invasive surgery for local excision of benign and malignant rectal neoplasia: outcomes from 200 conse-cutive cases with midterm follow up. Ann Surg 2018; 267:910–916.
159. Leggett B, Whitehall V. Role of the serrated pathway in colorectal cancer pathogenesis. Gastroenterology 2010; 138:2088–100.
160. Lester SC. Manual of Surgical Pathology. Elsevier Inc 2006; 420, 425-427, 448-450.
161. Levin B, Lieberman DA, McFarland B et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous Polyps: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. CA Cancer J Clin 2008; 58(3):130-160.
162. Levine AJ, Momand J, Finlay CA. The p53 tumor suppressor gene. Nature 1991; 351:453-6.
163. Li Z, Zhao X, Bai S et al. Proteomics identification of cyclophilin a as a potential prognostic factor and therapeutic target in endometrial carcinoma. Mol Cell Proteomics 2008; 7(10):1810- 1823.

164. Liao Z, Boileau THM, Erdman JW, Clinton SK. Interrelationships among angiogenesis, proliferation and apoptosis in the tumor microenvironment during N-methyl-N-nitrosourea androgen-induced prostate carcinogenesis in rats. Carcinogenesis 2002; 23(10): 1701-1712.
165. Lidgren A, Hedberg Y, Grankvist K, Rasmuson T, Vasko J, Ljungberg B. The expression of hypoxia-inducible factor 1alpha is a favorable independent prognostic factor in renal cell carcinoma. Clin Cancer Res 2005; 11: 1129-1135.
166. Lieberman D. Progress and challenges in colorectal cancer screening and surveillance. Gastroenterology 2010; 138:2115–26.
167. Liedke GS, da Silveira HE, da Silveira HL, Dutra V, de Figueiredo JA. Influence of voxel size in the diagnostic ability of cone beam tomography to evaluate simulated external root resorption. J Endod 2009; 35:233-235.
168. Lin AY. Open low anterior resection. In: JW Fleshman, Mutch MG, Birnbaum EH, Kodner IJ, Hunt SR, Safar B, eds. Atlas of Surgical Techniques for the Colon, Rectum, and Anus. Philadelphia, PA: Saunders 2013; 156–177.
169. Lingala SG, Sutton BP, Miquel ME, Nayak KS. Recommen-dations for real-time speech MRI. J Magn Reson Imaging 2016; 43(1):28–44.
170. Liu L, Yang L, Jin E, Wang Z, Yang Z. Effect of gadolinium contrast-enhanced T1-weighted magnetic resonance imaging for detecting extramural venous inva- sion in rectal cancer. Abdom Radiol (NY) 2016; 41(9):1736–1743.
171. Liu TS, Owsley JQ. Long-term results of face lift surgery: patient photographs compared with patient satisfaction ratings. Plast Reconstr Surg 2012; 129(1):253-262.
172. Lofthag-Hansen S, Thilander-Klang A, Gröndahl K. Evaluation of subjective image quality in relation to diagnostic task for cone beam computed tomography with different fields of view. Eur J Radiol 2011; 80:483-488.
173. Loubele M, Bogaerts R, Van Dijck E, Pauwels R, Vanheusden S, Suetens P, Marchal G, Sanderink G, Jacobs R. Comparison between effective radiation dose of CBCT and MSCT scanners for dentomaxillofacial applications. Eur J Radiol 2009; 71:461-468.
174. Loukas M, Youssef P, Gielecki J, Walocha KN, Tubbs RS. History of cardiac anatomy: a comprehensive review from the egyptians to today. Clinical Anatomy 2016; 29:270-284.
175. Ludlow JB, Ivanovic M. 2008. Comparative dosimetry of dental CBCT devices and 64-slice CT for oral and maxillofacial radiology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106:106-114.
176. Ma YL, Peng P, Zhang WJ, Liu L, Huang HL. Immunohistochemical analysis revealed CD34 and Ki67 protein expression as significant prognostic factors in colorectal cancer. Med Oncol 2010; 27(2):304-309.
177. Macchi V, Tiengo C, Porzionato A, Stecco C, Galli S, Vigato E, Azzena B, Parenti A, De Caro R. Anatomo-radiological study of the superficial musculo-aponeurotic system of the face. Ital J Anat Embryol 2007; 112(4):247–253.
178. Macchi V, Tiengo C, Porzionato A, Stecco C, Vigato E, Parenti A, Azzena B, Weiglein A, Mazzoleni F, De Caro R. Histotopographic study of the fibroadipose connective cheek system. Cells Tissues Organs 2010; 191(1):47–56.

179. Mah P, Reeves TE, McDavid WD. Deriving Hounsfield units using grey levels in cone beam computed tomography. Dentomaxillofac Radiol 2010; 39: 323-335.
180. Mainka A, Platzek I, Mattheus W, Fleischer M, Müller AS, Mürbe D. 2017. Three-dimensional vocal tract morphology based on multiple magnetic resonance images is highly reproducible during sustained phonation. J Voice 2017; 31:504e11-504e20.
181. Massad LS. Preinvasive disease of the cervix. In: DiSaia, Creasman, Mannel, McMee- kin, Mutch, eds. Clinical gynegologic oncology. Ed 9. Philadelphia, PA: Elsevier 2018; 1–19.
182. Mazzucchelli R, Montironi R, Santinelli A, Lucarini G, Pugnaloni A, Biagini G, Vascular endothelial growth factor expression and capillary architecture in high-grade PIN and prostate cancer in untreated and androgen-ablated patients. Prostate 2000; 45:72-79.
183. McDougall SR, Anderson ARA, Chaplain MAJ, Sherratt JA, Mathematical modelling of flow through vascular networks: implications for tumor-induced angiogenesis and chemotherapy strategies. Bull Math Biol 2002; 64(4): 673–702.
184. Mcleod HL, Murray GI. Tumour markers of prognosis in colorectal cancer. Br J Cancer 1999; 79:191-203.
185. Mendelson BC. Extended sub-SMAS dissection and cheek elevation. Clin Plast Surg 1995; 22(2):325–339.
186. Meningaud JP, Bertolus C, Bertrand JC. Parotidectomy: assessment of a surgical technique including facelift incision and SMAS advancement. J Craniomaxillofac Surg 2006; 34(1):34–37.
187. MERCURY Study Group. Diagnostic accuracy of preoperative magnetic resonance imaging in predicting curative resection of rectal cancer: prospective observational study. BMJ 2006; 333(7572):779.
188. MERCURY Study Group. Extramural depth of tumour invasion at thin- section MR in patients with rectal cancer: results of the MERCURY study. Radiology 2007; 243(1):132-9.
189. Messenger DE, Driman DK, Kirsch R. Developments in the assessment of venous invasion in colorectal cancer: implications for future practice and patient outcome. Human Pathology 2012; 43(7):965-973.
190. Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. Am J Surg Pathol 2005; 29(1):52-68.
191. Milliaris D, Kamas A, Kalekou H. Angiogenesis in invasive breast carcinoma: is it associated with parameters of prognostic significance? Histopath 1995; 26:165-169.
192. Mirnezami R, Chang GJ, Das P, Chandrakumaran K, Tekkis P, Darzi A et al. Intraoperative radiotherapy in colorectal cancer: systematic review and meta- analysis of techniques, long-term outcomes, and complications. Surg Oncol 2013; 22(1):22e35.
193. Mitz V, Peyronie M. The superficial musculoaponeurotic system (SMAS) in the parotid and cheek area. Plast Reconstr Surg 1976; 58:80-88.
194. Moore KL, Dalley AF, Agur AMR. Anatomia Orientada para a Clínica, VIIth edition. Ed. Nova Guanabara, 2014, ISBN: 9788527725170.

195. Mor N, Blitzer A. Functional Anatomy and Oncologic Barriers of the Larynx. Otolaryngologic Clinics of North America 2015; 48:533-545.
196. Mureşan R, Cosgarea M. Phoniatry – history and perspective. Int J Hum Voice 2013; 2(1):36–42.
197. Naitoh M, Hiraiwa Y, Aimiya H, Ariji E. Observation of bifid mandibular canal using cone- beam computerized tomography. Int J Oral Maxillofac Implants 2009; 24: 155-159.
198. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Rectal Cancer. V3. 2018. In. Version 3.2018 ed. NCCN.org2018.
199. Nieto Y, Woods J, Nawaz F et al. Prognostic analysis of tumour angiogenesis, determined by microvessel density and expression of vascular endothelial growth factor, in high-risk primary breast cancer patients treated with high-dose chemotherapy. Br J Cancer 2007; 97(3):391-397.
200. Nomura Y, Watanabe H, Honda E, Kurabayashi T. Reliability of voxel values from cone- beam computed tomography for dental use in evaluating bone mineral density. Clin Oral Implants Res 2010; 21:558-562.
201. O'Brien JX, Justin X, Rozen WM, Warren M, Whitaker IS, Ashton MW. Lore's fascia and the platysma-auricular ligament are distinct structures. J Plast Reconstr Aesthet Surg 2018; 65(9):241-245.
202. Oguz H, Hess M, Klein AM. Phoniatrics. Biomed Res Int 2015; 156014.
203. Ottaviano G, Cappellesso R, Mylonakis I, Lionello M, Favaretto N, Giacomelli L, Spoladore C, Marchese-Ragona R, Marino F, Staffieri A. Eur Arch Otorhinolaryngol 2015; 272(11):3367.
204. Owsley JQ Jr. SMAS-platysma facelift. A bidirectional cervicofacial rhytidectomy. Clin Plast Surg 1983; 10(3):429–440.
205. Park JS, Jang YJ, Choi GS et al. Accuracy of preoperative MRI in predicting pathology stage in rectal cancers: node-for-node matched histopathology validation ofMRI features. Dis Colon Rectum 2014; 57(1):32–38.
206. Park R. An Epitome of the History of Medicine. 2nd Ed. Philadelphia: Davis FA Company Publishers 1901; p 1–29.
207. Passavant PG: Über die Verschliesseng des Schlundes beim Sprechen. Virch ArchVolume 1932; 46: 1-32.
208. Patel S, Bhardwaj R, Parmar P, Vaniya VH. Morphometric characteristic of thyroid cartilage in Gujarat region: a cadaveric study. J Res Med Den Sci 2016; 4(2):150–154.
209. Paulsen F, Tillmann B. Functional and clinical anatomy of the posterior insertion of the human vocal ligament. Eur Arch Otorhinolaryngol 1997; 254:442-448.
210. Pavlopoulos PM, Konstantinidou AE, Agapitos E, Kavantzas N, Nikolopoulou P, Davaris P. A morphometric study of neovascularization in colorectal carcinoma. Cancer 1998; 83(10):2067-2075.
211. Pearl RM, Jhonson D. The vascular supply to the skin: an anatomical and physiological reappraisal- Part II. Ann Plast Surg 1983; 11(3):196-205.
212. Pecorelli S, Zigliani Z, Odicino F. Revised FIGO staging for carcinoma of the cervix. Int J Gynaecol Obstet 2009; 105:107.

213. Pensler JM, Ward JW, Parry SW. Superficial musculoaponeurotic system in the upper lip: an anatomic study in cadavers. Plast Reconstr Surg 1985; 75:488-494.
214. Persaud TVN, Loukas M, Tubbs RS. A history of human anatomy. 2nd Ed. Illinois: Charles C. Thomas 2014; p 27–30.
215. Pezzella F, Micklem K, Turley H, Pulford K, Jones M, Kocialkowski S, Delia D, Aiello A, Bicknell R, Smith K, Harris AL, Gatter KC, Mason DY. Antibody for detecting p53 protein by immunohistochemistry in normal tissues. J Clin Pathol 1994; 47:592-6.
216. Pfaendler KS, Wenzel L, Mechanic MB, Penner KR. Cervical cancer survivorship: long-term quality of life and social support. Clin Ther 2015; 37:39–48.
217. Pickhardt PJ, Choi JR, Hwang I et al. Computed tomographic virtual colonoscopy to screen for colorectal neoplasia in asymptomatic adults. N Engl J Med 2003; 349(23):2191-2200.
218. Pidgeon TE, Boca R, Fatah F. A technique for facial reanimation: the partial temporalis muscle-tendon transfer with a fascia lata sling. J Plast Reconstr Aesth Surg 2017; 70: 313-321.
219. Piquet J, Decroix G: Etude éxperimentale peropératoire du róle de la pression sous-glottique sur la vibration des cordes vocales. Comptes Rend. Acad. Sc. Paris 1956; 242: 1223-1225.
220. Poon FW, McDonald A, Anderson JH et al. Accuracy of thin section magnetic resonance using phasedarray pelvic coil in predicting the T-staging of rectal cancer. Eur J Radiol 2005; 53:256-62.
221. Portmann G, Robin JL: L'electrodomyographie des cordes vocales de l'homme pendant la phonation. C. R. Soc. Biologie 1955; 149:1783-1787.
222. Potack J, Itzkowitz SH. Practical advances in stool screening for colorectal cancer. J Natl Compr Canc Netw 2010; 8:81–92.
223. Prorok PC, Andriole GL, Bresalier RS et al. Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial. Control Clin Trials 2000; 21(6):S273–S309.
224. Quer M, Vander Poorten V, Takes RP, Silver CE, Boedeker CC, de Bree R, Rinaldo A, Sanabria A, Shaha AR, Pujol A, Zbären P, Ferlito A. Surgical options in benign parotid tumors: a proposal for classification. Eur Arch Otorhinolaryngol 2017; 274(11):3825–3836.
225. Ramos BD. Phoniatry: otorhinolaryngology expands its limits. Braz J Otorhinolaryngol 2015; 81(6):581–582.
226. Razi T, Niknami M, Ghazani FA. Relationship between Hounsfield Unit in CT Scan and Gray Scale in CBCT. J Dent Res Dent Clin Dent Prospects 2014; 8:107–110.
227. Riddle RH, Petras RE, Williams GT, Sobin LH. Atlas of tumor pathology, tumors of the intestines. Armed Forces Institute of Pathology, Washington DC, 2003.
228. Riihimaki M, Hemminki A, Sundquist J, Hemminki K. Patterns of metastasis in colon and rectal cancer. Sci Rep 2016; 6:29765.
229. Roberts JA, Drage NA, Davies J, Thomas DW. Effective dose from cone beam CT examinations in dentistry. Br J Radiol 2009; 82:35-40
230. Rockey DC. Computed tomographic colonography: current perspectives and future directions. Gastroenterology 2009; 137:7-14.

231. Romani AA, Borghetti AF, Del Rio P, Sianesi M, Soliani P. The risk of developing metastatic disease in colorectal cancer is related to CD105-positive vessel count. J Surg Oncol 2006; 93(6):446.
232. Rombouts AJMM, Koh CE, Young JM et al. Does Radiotherapy of the Primary Rectal Cancer Affect Prognosis After Pelvic Exenteration for Recurrent Rectal Cancer? Dis Colon Rectum 2015; 58(1):65-73. doi:10.1097/DCR.0000000000000213.
233. Rothwell PM, Fowkes GR, Belch JF, et al. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomized trials. Lancet 2010.
234. Ruivo J, Mermuys K, Bacher K, Kuhweide R, Offeciers E, Casselman JW. Cone beam computed tomography, a low-dose imaging technique in the postoperative assessment of cochlear implantation. Otol Neurotol 2009; 30: 299-303.
235. Sabo E, Boltenko A, Sova Y, Stein A, Kleinhaus S, Resnick MB. Microscopic Analysis and Significance of Vascular Architectural Complexity in Renal Cell Carcinoma, Clinical Cancer Res 2001; 7: 533-537.
236. Sagar PM, Pemberton JH. Surgical management of locally recurrent cancer. Br J Surg 1996; 83:293-304.
237. Sakuragi N. Nerve-sparing radical hysterectomy: time for a new standard of care for cervical cancer? J Gynecol Oncol 2015; 26:81–82.
238. Salerno G, Daniels IR, Moran BJ, Wotherspoon A, Brown G. Clarifyng margins in the multidisciplinary management of rectal cancer: the MERCURY experience. Clin Radiol 2006; 61:916-23.
239. Samdani T, Garcia-Aguilar J. Imaging in rectal cancer: magnetic resonance imaging versus endorectal ultrasonography. Surg Oncol Clin N Am 2014; 23(1):59–77.
240. Santoni R, Bucciolini M, Cionini L, Ciraolo L, Renzi R. Modifica- tions of relaxation times induced by radiotherapy therapy in cervi- cal carcinoma: preliminary results. Clin Radiol 1987; 38(6):569-573.
241. Sauer R, Liersch T, Merkel S, Fietkau R, Hohenberger W, Hess C, Becker H, Raab HR, Villanueva MT, Witzigmann H, Wittekind C, Beissbarth T, Rodel C. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: results of the German CAO/ARO/ AIO-94 randomized phase III trial after a median follow-up of 11 years. J Clin Oncol 2012; 30:1926-33.
242. Scarfe WC, Farman AG. What is Cone-Beam CT and How Does it Work. Dent Clin N Am 2008; 52:707–730.
243. Schmalfuss IM, Neri E, Bartolozzi C, Rijpkema M, Kaanders J. Recent advances in laryngeal imaging. In: Hermans R (ed). Imaging of the larynx. Series "Medical Radiology (DiagnosticImaging)", Springer Verlag, Berlin–Heidelberg 2003; p.157–184.
244. Sebag-Montefiore D, Stephens RJ, Steele R, Monson J, Grieve R, Khanna S, Quirke P, Couture J, de Metz C, Myint AS, Bessell E, Griffiths G, Thompson LC, Parmar M. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, m randomized trial. Lancet 2009; 373:811-20.
245. Seikel JA, King DW, Drumright DG. Anatomy & physiology for speech, language and hearing. 4th edition, Delmar Cengage Learning, New York 2009; p.223–266.

246. Shihab OC, Taylor F, Salerno G, Heald RJ, Quirke P, Moran BJ, Brown G. MRI predictive factors for long-term outcomes of low rectal tumours. Ann Surg Oncol 2011; 18(12):3278-3284.
247. Siegel RL, Miller KD, Jemal A. Cancer statistics 2018. CA Cancer J Clin. 2018; 68(1):7-30.doi:10.3322/caac.21442.
248. Siemionow M, Papay F, Kulahci Y, Djohan R, Hammert W, Hendrickson M, Zins J. Coronal-posterior approach for face/ scalp flap harvesting in preparation for face transplantation. J Reconstr Microsurg 2006; 22(6):399–405.
249. Slater A, Halligan S, Taylor SA, Marshall M. Distance between the rectal wall and mesorectal fascia measured by MRI: effect of rectal distension and implications for preoperative prediction of a tumor-free circumferential resection margin. Clin Radiol 2006; 61(1):65-70.
250. Small Jr W, Bacon MA, Bajaj A, et al. Cervical cancer: a global health crisis. Cancer 2017; 123:2404–2412.
251. Smith N, Brown G. Preoperative staging of rectal cancer. Acta Oncol 2008; 47:20-31.
252. Smith NJ, Shihab O, Arnaout A, Swift RI, Brown G. MRI for detection of extramural vascular invasion in rectal cancer. AJR Am J Roentgenol 2008; 191(5):1517-22.
253. Sokmen S, Sarioglu S, Fuzun M, Terzi C, Kupelioglu A, Aslan B. Prognostic significance of angiogenesis in rectal cancer: a morphometric investigation. Anticancer Res 2001; 21(6B): 4341-4348.
254. Standring S (ed). Gray's anatomy: the anatomical basis of clinical practice. 41st edition, Elsevier, 2016, 955–987.
255. Standring, S., 2016. Gray's Anatomy, 41st ed. Elsevier/Churchill Livingstone, New York, pp. 404–708.
256. Stathopoulos P, Igoumenakis D, Smith WP. Partial superficial, superficial, and total parotidectomy in the management of benign parotid gland tumors: a 10-year prospective study of 205 patients. J Oral Maxillofac Surg 2018; 76(2):455–459.
257. Stevenson ARL, Solomon MJ, Brown CSB et al. Disease-free Survival and local recurrence after laparoscopic-assisted resection or open resection for rectal can- cer: the Australasian laparoscopic cancer of the rectum randomized clinical trial. Ann Surg Epub ahead of print 2018.
258. Stuzin JM, Baker TJ, Gordon HL. The relationship of the superficial and deep facial fascias: relevance to rhytidectomy and aging. Plast Reconstr Surg 1992; 89(3): 441–449.
259. Sundberg J. Vocal tract resonance in singing. The Nat Ass of Teachers of Singing J 1988; 44:11-31.
260. Suomalainen A, Kiljunen T, Käser Y, Peltola J, Kortesniemi M. Dosimetry and image quality of four dental cone beam computed tomography scanners compared with multislice computed tomography scanners. Dentomaxillofac Radiol 2009; 38:367-378.
261. Talbot IC, Ritchie S, Leighton MH, Hughes AO, Bussey HJ, MorsonBC. The clinical significance of invasion of veins in cancer of the rectum. Br J Surg 1980; 67(6): 439-42.
262. Tamplen M, Knott PD, Fritz MA, Seth R. Controversies in parotid defect reconstruction. Facial Plast Surg Clin North Am 2016; 24(3):235–243.

263. Taylor F, Swift R, Blomqvist L, Brown G. A systemic approach to the inter- pretation of preoperative staging MRI for rectal cancer. AJR Am J Roentgenol 2008;191:1827-35.
264. Taylor FG, Quirke P, Heald RJ et al. Preoperative magnetic resonance imaging assessment of circumferential resection margin predicts disease-free survival and local recurrence: 5-year follow-up results of the MERCURY study. J Clin Oncol 2014; 32(1):34–43.
265. Taylor GI, Palmer JH. The vascular territories(angiosomes) of the body: Experimental study and clinical applications. Br J Plast Surg 1987; 40:113-141.
266. Tessier P. Le lifting facial sous-perioste. Ann Chir Plast Esthet 1989; 34:193-198.
267. Thaller SR, Kim S, Patterson H, Wildman M, Daniller A. The submuscular aponeurotic system (SMAS): a histologic and comparative anatomy evaluation. Plast Reconstr Surg 1990; 86(4):690–696.
268. Thiagarajan B. Assessment of vocal fold phonatory function. A review. Otolaryngol Online J 2015; 5:1-5.
269. Thiis-Evensen E, Hoff GS, Sauar J, Langmark F, Majak BM, Vatn MH: Population-based surveillance by colonoscopy: effect on the incidence of colorectal cancer. Telemark Polyp Study I Scand J Gastroenterol 1999; 34(4):414–20.
270. Tillmann B, Paulsen F. Functional and clinical anatomy of the anterior commissure. Adv Otorhinolaryngol Basel Karger 1995; 49:201-206
271. Torkzad MR, Pahlman L, Glimelius B. Magnetic resonance imaging (MRI) in rectal cancer: a comprehensive review. Insights Imaging 2010; 1:245-67.
272. Troja A, El-Sourani N, Abdou A, Antolovic D, Raab HR. Surgical options for locally recurrent rectal cancer—review and update. Int J Colorectal Dis 2015; 30(9):1157-1163.
273. Tubbs RS, Loukas M, Shoja MM, Ardalan MR, Oakes WJ. Richard Lower (1631–1691) and his early contributions to cardi- ology. Int J Cardiol 2008; 128:17–21.
274. US Preventive Services Task Force: Screening for colorectal cancer: US Preventive Services Task Force recommendation statement. Ann Behav Med 2008; 149:627-37.
275. Van den Berg J: Myoelastic-aerodynamic Theory of Voice production. Journal of Speech and Hearing Research 1958; 3: 227-244.
276. Van der Geest LG, Lam-Boer J, Koopman M, Verhoef C, Elferink MA, de Wilt JH. Nationwide trends in incidence, treatment and survival of colorectal can- cer patients with synchronous metastases. Clin Exp Metastasis 2015; 32(5):457–465.
277. Van Ramshorst GH, O'Shannassy S, Brown WE, Kench JG, Solomon MJ. A qualitative study of the development of a multidisciplinary case conference review methodology to reduce involved margins in pelvic exenteration surgery for recurrent rectal cancer. Color Dis 2018; 20(11):1004- 1013.
278. Van Rossum LG, Van Rijn AF, Laheij RJ et al. Random comparison of guaiac and immunochemical fecal occult blood tests for colorectal cancer in a screening population. Gastroenterol 2008; 135:82–90.
279. Viswanathan AN, Lee LJ, Eswara JR, Horowitz NS, Konstantinopoulos PA, Mirabeau- Beale KL, Rose BS, von Keudell AG, Wo JY. Complications of pelvic radiation in patients treated for gynecologic malignancies. Cancer 2014; 120:3870–3883.
280. Vos RR, Murphy DT, Howard DM, Daffern H. The Perception of Formant Tuning in Soprano Voices. J Voice 2018; 32:126.e2-126.e3,

281. Wang Y, Reed M, Wang P, Stenger JE, Mayr G, Anderson ME, Schwedes JF, Tegtmeyer P, p53 domains: identification and characterization of two autonomous DNA-binding regions. Genes Dev 1993; 7(12B):2575-2586.
282. Wassef M. Superficial fascial and muscular layers in the face and neck: a histological study. Aesthetic Plast Surg 1987; 11(1):171–176.
283. Whetzel TP, Mathes SJ. Arterial anatomy of the face: An analysis of vascular territories and perforating cutaneous vessels. Plast Reconstr Surg 1992; 89:591-603.
284. Winawer SJ, Zauber AG, Ho MN et al.: Prevention of colorectal-cancer by colonoscopic polypectomy. N Engl J Med 1993; 329(27):1977–81.
285. Winawer SJ: Colorectal cancer screening. Best Pract Res Clin Gastroenterol 2007; 21(6):1031-48.
286. Wong WK, Shetty S. Classification of parotidectomy: a proposed modification to the European Salivary Gland Society classification system. Eur Arch Otorhinolaryngol 2017; 274(8):3175–3181.
287. Wuerthner BA, Avila-Wallace M. Cervical cancer: screening, management, and prevention. Nurse Pract 2016; 41:18–23.
288. Yamawaki Y, Nishimura Y, Suzuki Y. Velopharyngeal closure and the longuscapitis muscle. Acta Otolarygolog (Stockh) 1996; 116:774–777.
289. Yamawaki Y, Nishimura Y, Sawada M, Shimidzu K. Dynamic MRI and velopharyngeal closure: preliminary report. J Jpn P R S 1994; 14:116-122.
290. Yousif NJ, Gosain A, Matloub HS, Sanger JR, Madiedo G, Larson DL. The nasolabial fold: an anatomic and histologic reappraisal. Plast Reconstr Surg 1994; 93:60-69.
291. Zigiotti GL, Liverani MB, Ghibellini D. The relationship between parotid and superficial fasciae. Surg Radiol Anat 1991; 13(4):293–300.