GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

ANATOMY WITHOUT FRONTIERS, A SYSTEMIC MORPHO-FUNCTIONAL APPROACH

- HABILITATION THESIS -

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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

SECTION I

ABSTRACT

The applying for improvement professional standards in the academic career is compulsory and ensures the quality of the educational system.The current standards required for academic and medical performance focus on the awareness of continuous updated training, the integration of modern teaching and researching methods to qualify for high educational level achievements.

Academic career is a complex challenge and relies on perseverance and desire for self-refinement, receptivity to new ideas and concepts, flexibility, dynamism and critical evaluation. It has significant impact upon the entire academic community and it must blend harmoniously with solid scientific knowledge, availability and pleasure to communicate with others, desire to be part of a team, capacity to create and coordinate functional team works, ability to identify and motivate human resources.

The Habilitation Thesis, which synthesizes my postdoctoral professional, academic and scientific activity is structured in three major sections, according to the The National Council for Attestation of University Titles, Diplomas and Certificates (CNATDCU) recommendations and criteria. The paper presents the overview of my pursuits in the interest domains. It is entitled "*Anatomy without frontiers, a systemic morpho-functional approach*" and presents my research in the field of anatomy and especially the clinical anatomy.

Regarding the already mentioned criteria I have structured the thesis into two main parts. Section I, the first part contains the Abstract of the Habilitation Thesis.

Section II, the second and last part contains a detailed description of the professional, scientific and academic achievements.

The last section is subdivided into three subsections: Chapter 1, 2 and 3. The first one consists of the abridgement of al my professional, scientific and academic achievements gained and succeeded after PhD study.

Chapter 2 highlights my scientific and professional achievements in order to reunite the main personal research on the three main study directions: superficial musculoaponeurotic system of the face and neck, canto voice morfofunctional features and neovasculogenesis phenomena in colorectal cancers.

Chapter 3 lists the most relevant referrence I have used for this thesis and for the articles included. A considerable number of this references are new ones, from the last 5 years, meaning that the subject and the themes from my thesis are current issues in anatomy.

Chapter 1 contains a brief overview of my professional, academic and scientific activities, where I reviewed my studies and the main research topics I have followed after my PhD thesis. It presents a summary of my research, teaching and medical work performed since the doctoral thesis graduation (2010) and contains detailed descriptions of the projects for future research. It is organised in four chapters as follows: academic activity and career overview, research projects and scientific activities, synopsis of the PhD thesis and international visibility.

I have described all my research domains and the projects which I have conducted, together withthe results of my academic work.

Chapter 2 unites under a systemic vission the most important results of my main research topic; they are materialized in three main study directions: superficial musculoaponeurotic system of the face and neck (SMAS), the anatomical background of the distinctive, particular vocal abilities in the canto voice, radiological, clinical anatomy and histological aspects of paraneoplastic neovasculogenesis processes in case of the colorectal cancers.

SMAS research direction came out ofmy doctoral study. The results of this study led to to deepen the knowledge aboutthe new concept of a superficial cervicofacial cover layer, with particular practical features.

The direction of study regarding the canto voice has derived from two conjuncted directions:comparative anatomy studies of the *morphofunctional mechanisms* that work together to determine *exceptional vocal abilities* are not fully known yet. Our findings in this area were decisive in determinating me to deepen my research of this vast field and I explored the upper segments of the phonatory apparatus through innovative and modern techniques.

The study of paraneoplastic neovasculogenesis processes has naturaly stemmed from myclinicalpractise that I have as a general surgery specialist and proctologist. Colorectal cancers are the most common digestive malignant pathologies in my medical field, with a growing incidence on the international scene.

In this manuscript I present all my research findings in these three main research directions that I have followed, starting with the scientific context of each of them and related results. This is compulsory because each research direction will be continued and represents a starting point for future research projects. These projects are detailed in the specific *2.4.5.* subchapters of this manuscript.

Chapter 3 includes a number of 256 bibliographic references which certify the current knowledge and discussions on the topics, the related articles and the thesis itself.

REZUMAT

Aplicarea standardelor profesionale în cariera academică este obligatorie și asigură creșterea calității sistemului educațional. Standardele actuale necesare pentru performanța academică și medicală se concentrează pe conștientizarea necesității instruirii și actualizării continue a cunoștințelor cât și pe integrarea metodelor moderne de predare și cercetare pentru a se califica pentru realizări de nivel înalt de educație.

Cariera academică este o provocare complexă și se bazează pe perseverență și dorință de auto-rafinament, receptivitate la idei și concepte noi, flexibilitate, dinamism și evaluare critică. Ea are un impact semnificativ asupra întregii comunități academice și trebuie să se armonizeze cu cunoștințele științifice solide, disponibilitatea și plăcerea de a comunica cu ceilalți, dorința de a face parte dintr-o echipă, capacitatea de a crea și coordona echipe de cercetare, capacitatea de a identifica și motiva resursa umană.

Teza de Habilitare sintetizează activitatea mea profesională, academică și științifică postdoctorală și este structurată în două mari secțiuni, conform recomandărilor și criteriilor Consiliului Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU).

Lucrarea prezintă o imagine de ansamblu asupra principalelor domenii de interes ale mele. Ea este intitulată "Anatomia fără frontiere, o abordare sistemică morfo-funcțională" și prezintă cercetarea mea în domeniul anatomiei și mai alesîn cel al anatomiei aplicate clinic.

În ceea ce privește criteriile deja menționate, am structurat teza în două părți principale.

Secțiunea I (Section I), prima parte conține Rezumatul tezei de habilitare.

Secțiunea a II-a (Section II), a doua și ultima parte a tezei, conține o descriere detaliată a realizărilor profesionale, științifice și academice.

Ultima secțiune este subîmpărțită în trei subsecțiuni: Capitolele 1, 2 și 3.

Prima (Chapter 1) se referă la reducerea performanțelor profesionale, științifice și academice obținute și reușite după studii de doctorat.

Capitolul 2 (Chapter 2) evidențiază realizările mele științifice și profesionale pentru a reuni principalele cercetări personale pe cele trei direcții principale de studiu pe care le am: sistemul musculoaponeurotic superficial al feței și gâtului, caracteristicile morfofuncționale vocale canto și fenomenele neovasculogenezei în cancerele colorectale.

Capitolul 3 (Chapter 3) enumeră cele mai relevante manuscrise de pe care le-am folosit până acum pentru cunoașterea fiecăruia dintre cele trei domenii principale de cercetare.

Capitolul 1 (Chapter 1) conține o scurtă trecere în revistă a activităților mele profesionale, academice și științifice, în care mi-am revizuit studiile și principalele teme de cercetare pe care le-am urmat după finalizarea studiului doctoral. Ea prezintă rezumatul cercetărilor, lucrărilor didactice și medicale efectuate de la prezentarea tezei de doctorat (2010) și până în prezent și conține descrieri detaliate ale proiectelor pe care le am în vedere pentru cercetări viitoare. Această parte a tezei este organizată în patru capitole după cum urmează: activitatea academică și cariera, proiecte de cercetare și activități științifice, sinteza tezei de doctorat și vizibilitatea internațională.

În această parte a manuscrisului am descris toate domeniile mele de cercetare și proiectele pe care le-am desfășurat, împreună cu rezultatele activității mele academice.

Capitolul 2 (Chapter 2) unește sub o viziune sistemică cele mai importante rezultate ale subiectelor principale de cercetare. Acestea se materializează în trei direcții primordiale de studiu: sistemul musculoaponeurotic superficial al feței și gâtului (SMAS), bazele anatomice ale abilităților vocale distincte, particulare în vocea de canto și anatomia radiologică, clinică și aspectele histologice ale proceselor neovasculogenezei paraneoplazice în cazurile de cancer colorectal.

Direcția de cercetare a SMAS a reieșit din studiul meu de doctorat. Rezultatele acestui studiu m-au condus la aprofundarea cunoștințelor despre noul concept al unui strat superficial unic de acoperire cervicofacial, cu particularități practice deosebite.

Direcția de studiu privind vocea decanto a derivat din două direcții conjuncte: studiile comparative de anatomie ale *mecanismelor morfofuncționale* care se autodetermināîn vederea dezvoltării unor*abilitățile vocale excepționale*și care nu sunt încă pe deplin cunoscute.

Rezultatele cercetării noastre în acest domeniu au fost decisive în a mă determinasă aprofundez cercetarea acestui vast domeniu și am început-o explorând segmentele superioare ale tractului vocal prin tehnici de studiu inovatoare și moderne.

Studiul proceselor de neovasculogeneză paraneoplazică a derivat în mod natural din practica mea clinică pe care o am ca specialist în chirurgie generală și proctolog. Tumorile colorectale sunt cele mai frecvente patologii digestive maligne din domeniul meu medical, cu o incidență crescândă pe scena internațională.

În acest manuscris vă prezint toate rezultatele și concluziile cercetării mele în cele trei direcții principale de studiu pe care le-am urmărit, începând cu contextul științific al fiecăreia și discuțiile asociate. Acest lucru este obligatoriu deoarece fiecare direcție de cercetare va fi continuată și reprezintă un punct de plecare pentru viitoarele proiecte de cercetare. Aceste proiecte sunt detaliate în subcapitolele *2.4.5.* ale acestui manuscris.

Capitolul 3 include un număr de 256 de referințe bibliografice care certifică în prezent cunoștințele legate de momentul actual al cunoașterii și discuțiile pe aceste teme, legate de articolele asociate tezei însăși.

SECTION II

CHAPTER 1. PROFESSIONAL, SCIENTIFIC AND ACADEMIC ACHIEVEMENTS

Education is a must for development of the individual and implicitly for that of the society. Each of us is encouraged to make use of the opportunities offered by lifelong education. Once one reaches academical education level, one must have aquired knowledge and skills, individual characteristics required by the chosen profession and motivation. While some aspects can be present from birth, such as oratory and pedagogic talents, others need to be tought. Both have to be improved with exercise, steady continuous works, taking into account the reference models we have.

Career development focuses on the following aspects: experience and skils gained with didactic and professional activity, results obtained, future plans; research activity with results contributing in its specific field and future objectives.

1.1. **Professional achievements**

In 2004 I was admitted as a general surgery resident by order of the Romanian Minister of Health. In 2011, I finished this specialty with the title of General Surgery General Practitioner (Order of the Ministry of Health No. 9546 of 02.05.2011). From 2012 until now, I have been working at the First Surgery Clinic of the Emergency University Clinical Hospital ”St. Spiridon”, with clinical integration.

The experience gained from this side of my professional development has helped me in my teaching career and has given me the opportunity to "learn living anatomy". Another cornerstone in my career development and deepening of my personal knowledge of clinical and applied anatomy was the possibility to perform a comparative dissection-surgery study. The fact that currently - between the topics of the exam for specialist in general surgery - 3 topics are pure anatomy has strengthened my conviction of the importance of the steps I took towards my career development.

Based on this experience , I followed the postgraduate course of Digestive Diagnostic Endoscopy and succeeded the attestation exam held in the 2nd Medical Section of St. Spiridon Iasi Hospital (I series, no 22430, 1.11.2012-30.04. 2013).

Meanwhile, I registered in January 2019 in another surgical specialty in the field of Otorhinolaryngology. I have done so, as I am looking forward to open up new ways to deepen the research of the morpho-functional substrate of the singing voice.

1.2. **Academic activity**

Didactic positions gained through contest:

- Lecturer – from February 2014 until present, Morphofunctional Sciences I Department, Discipline of Anatomy, Faculty of Medicine, ”Grigore T. Popa” University of Medicine and Pharmacy Iași.

- Assistant professor – from October 2007 until February 2014, Morphofunctional Sciences I Department, Discipline of Anatomy, Faculty of Medicine, ”Grigore T. Popa” University of Medicine and Pharmacy Iași.
- Associate instructor – fromnovember2002 until October 2007, Discipline of Anatomy, Faculty of Medicine, ”Grigore T. Popa” University of Medicine and Pharmacy Iași.

From the begining, my didactic career fused with scientific research activity.

I started my didactic activity in October 2002 at the Anatomy Department of the Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy Iasi. A month later I sustained the exam, and since then I have been taking part in the practical classes of Anatomy. Up until 2004, I participated on a voluntary basis, as I had to complete the intership year, according to the legislation at the time. On 01.01.2004 I have been assigned to the teaching staff of the Faculty of Medicine of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi, within the Anatomy III Discipline, under the direction of Professor Doctor Doina Lucia Frîncu.

Between 2002 and 2018, I taught both courses and laboratory at the Department of Anatomy and Embryology for all the study directions - Medicine, Dentistry and Pharmacy, in both romanian and english study programs.

To further develop my psycho-pedagogical abilities and specific methodical skills required for academical education, I followed a post-university internship course for Didactic Personalization named „Didactica Universitara”,organized by U.M.F.”Gr. T. Popa” Iasi, in 2004-2005.

My promotion to the title of Lecturer at the same Department has enabled the opportunity of teaching the courses of Anatomy and Embryology to the 1st and 2nd academic year, for both English and Romanian series of students. I have focused my courses on student involvment while concurrently developing my student's academic self expression and presentation skills.

I presentedthemes based on the images acquired from personally performed dissections and on the synthesis of the materials from the discipline syllabus. To help the students understand the functionality of the studied structures, I have taken a pragmatic approach, making real life clinical multidisciplinary connections.

During my career, I was an invited lecturer for the postgraduate courses at the First Surgery Clinic of "Sf. Spiridon" Iasi where I lectured about the anterior lateral abdominal wall and extrahepatic bile ducts.

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

I also participated as an invited lecturer at 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 began my scientific research projects simultaneously with my my enrollment in the PhD studies. I was admitted to be PhD training at the ‘Grigore T. Popa’ University of Medicine and Pharmacy Iaşi in 2004 and in 2010 I obtained the title of Doctor in Medical

Sciences, (O.M. 6562, from 06.06.2011) – with the thesis entitled: "Morphogenetic, anatomical, clinical and functional considerations on musculo-aponeurotic cervicofacial system", also known as SMAS, scientifically coordinated by Prof. Doina Lucia Frîncu, MD, PhD. During the preparation of the PhD thesis, I updated and improved series of anatomical dissection techniques of cervico-facial structures

I chose an ambitous theme because the study of the face raises great ethical impediments. The most difficult aspect was the collection of superficial facial tissue specimens (from the skin to the mimic muscles) from cadavers, but particularly from patients. The original character of the thesis is supported by the complex research directions, starting from the embryological study, continuing with the pure anatomical study (quantitative and qualitative macro- and microanatomical) and including radiological and clinical studies.

The objectives proposed and achieved throughout my PhD research project have a strong practical and scientific impact. The practical impact primarily regards the health status of the population with respect to the risk factors involved in the processes of aging changes in the face and the possibilities of delaying them. It should be emphasized that the study has strong implications in facial rejuvenation surgery, bringing the anatomical evidence that could form the basis for future operative techniques.

The theme that I approached gives new research perspectives on facial antigravity support mechanisms, on mapping the facial nerve tracts and on a possible new approach to the facial regions.

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The methods used for this study have yielded reproducible results both under laboratory conditions and in current medical and didactical practice.

The core of the thesis adresses the existence of SMAS in different regions of the face, with it’s subtypes. This study has a great practical significance, paving the way for new interventional techniques in plastic and reconstructive surgery of the face and neck. It contains valuable information in helping understandthe phenomena of “the aging face”. The foundation of a new anatomical, practical and teaching approaches have been also analysed.

After I graduated the PhD program I have continued studying the SMAS and I have approached new research areas. I am particularly concerned in fathoming the anatomical background of voice opera and the vascular apparatus of both colorectal and cervical cancers.

I also adressed multiple other research topics, of which I wish to highight my involvment in the research on colorectal cacinomas and later, on a third and most important topic, the morfofunctional, clinical and radiologic anatomy of the vocal tract. Combining both anatomist and surgeon careers, I gained experience in radiological anatomy, clinical and paraclinical evaluation of patients.

Research projects

The project titled ”Morphofunctional Study of the Higher Formant of the Voice” is a research project that has fulfilled the requirements and obtainedthe agreement of the Ethics Committee as well as the Scientific Research Committee of UMF "Grigore T. Popa"Iasi.

International visibility

In all my career years, I constantly strived to increase my international visibility by:

- ✓ actively participating in international congresses
- ✓ publishing articles in academic journals with good impact factor
- ✓ participating in experience exchanges with other centers through the Erasmus + Mobility of Teachers Program, from abroad
- ✓ h-index 9, with 114 citations, according to data from the Web of Science website
- ✓ Using hosting services such as SlideShare to disseminate research results
- ✓ Using the ORCID platform that provides a persistent identity for humans

1.4. **Achievements in scientific publications field**

Within each research direction mentioned above, my research activity has led to a scientific portfolio of published papers (specialized books and teaching materials, in extenso and abstracts, articles in ISI Web of Knowledge indexed journals and other journals recognized in international databases), and to my participation at national and international congresses (with orally communicated papers or posters):

- 4 books as first author;
- 25 *in extenso* articles in ISI Web of Knowledge - indexed journals (22 as main author);
- 25 *in extenso* articles in journals recognized in international databases (23 as main author);
- 1 *in extenso* article in ISI Proceedings at international scientific manifestations, as main author
- 114 citations in ISI Web of Knowledge- indexed journals;
- 30 papers in the conference books with ISBN;
- 8 poster presentations at international scientific manifestations;

CHAPTER 2.
SCIENTIFIC AND PROFESSIONAL ACHIEVEMENTS

Most of my research career is materialized in three main study research directions:

A. SMAS
B. The anatomical substrate of the distinctive vocal abilities in the canto voice
C. Radiological, clinical anatomy and histological aspects of paraneoplastic neovasculogenesis processes in case of the colorectal cancers.

The first research direction emerged during my doctoral study. The results of this study led to the researching of the new concept of a superficial cervicofacial covering, with its particular practical importance in plastic and reconstructive surgery.

The second research direction of study has derived from two converging topics: comparative anatomy studies and not yet fully understood, morphofunctional mechanisms, working together to determine exceptional vocal abilities

The comparative anatomical study idea of the vocal tract started from a research made by British veterinary surgeon Mark Evans. He conducted his research on racing horses and the medical problems they face. In 2000, at *Animal Planet's Hi-Tech Vets,* he presented the results of a study that explained why descendants of highly performing horses do not meet expectations. The English physician adapted a videolaringoscope of these horses, with which he could record their glotic activity during a race. Thus, he demonstrated that some horse specimens present a glottic spasm upon increasing their required strain intensity, which disabled them to achieve the desired performance their parent was able to.

Dr Mark Evans' presentation was decisive in determinating me to approach this vast research field I started by exploring the upper segments of the vocal tract through innovative and modern techniques.

The third research direction of study has naturaly stemmed from my medical practice as a general surgery specialist and proctologist. Colorectal cancers are the most common malignant pathologies in my medical field, with a growing incidence on the international scene. Anatomical research in the past 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. The use of modern radiological techniques in the evaluation of paraneoplastic vasculogenesis processes 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 follow-up of patients is a must in the effort to develop a protocol for the management of patients with malignant tumors.

This thesis will further present all my research findings in the above three main research directions that I have followed, starting with the scientific context of each one of them and related results. I feel it is necessary to do so, as each research direction will most certanly be continued in the immediate and distant future. Furthermore, each represents a starting point for future research projects. These projects are detailed in the specific chapter of this manuscript.

2.1. ANATOMICAL SUBSTRATE OF SUPERFICIAL MUSCULOAPONEUROTIC SYSTEM OF THE FACE

2.1.1. State of the art

The first description of SMAS was made by Mitz and Peyronie in 1976, after dissecting the parotid and cheek regions (buccal after NA) (Mtiz, 1976), which led to a controversy between the surgeons and anatomists. This structure was initially recognized only by the surgeons, who use it in various facelift techniques (Stuzin et al., 1992, Tessier 1989, Moss et al., 2000, Mendelson 2001). The superficial layers of the face are under the permanent action of gravitational force in a unique manner. This is due on one hand to the bipedal resort, and on the other to the expression of the human specific functions (mimics, socialization).

The microscopic anatomy and the biomechanics of the superficial facial surface structure include a set of shapes which interpose on the gravitational force, on the aging and the phenomenon of “falling” of the face. These structures consist of the continuity of superficial fascia with the epicranial fascia, adhesions and fixing perizygomatic fixation ligaments, of the adhesion of the superficial structures to the peri- and inter-orbital muscles, and of the presence of fibro-adipose tissue regionally organized, with a role in facilitating the dragging during contractions (Stuzin et al., 1992, Mendelson 1997, Gola 2005).

We intended to identify the anatomical formations, defined as sustentaculum facies, which hold an important function in supporting the superficial layers. We also intended to explore the possibilities of various techniques in rhytidectomy.

Facial SMAS extends laterally and posteriorly up to the parotid region. At this level, the parotid fascia is forming a capsule around the gland, together with the masseteric fascia. It encloses the parotid gland, its excretory duct, as well as the branches of the facial nerve. Commonly, is considered that the parotid fascia proceeds from the superficial layer of the deep cervical fascia, which then splits to cover the gland (Tamplen et al. 2016).

It is named parotid fascia as 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, the lamina profunda that covers the stylohyoid, the styloglossus and stylopharyngeus muscles (Berry and Davies 2010).

The superficial layer attaches to the zygomatic arch and mandibular body. One of the goals of my research was to objectify the origin of the superficial parotid fascia, considered to be a duplication of the deep fascia, by analyzing its quantitative and qualitative anatomy.

Plastic surgeons (Berry and Davies 2010) 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 is nowadays considered to form a muscular aponeurotic cervico-facial unit (Khan, 2014). The importance of our study derives from the practical utility of the SMAS concept, which we showed that is continued at parotid level. This finding allows surgical techniques to minimize postoperative incidents. Thus, the main surgical approach to parotid tumour based on the SMAS concept is extracapsular lumpectomy with SMAS flap (Bonanno and Casson 1992, Giannone et al., 2008, Dell'Aversana et al., 2015).

Essentialy, preserving a flap of SMAS in parotid interventions, can reduce the risk of parotid Frey syndrome (gustatory sweating). This technique, also minimizes the risk of postoperative infection and allows reconstruction of the parotid lodge (Lee et al., 2017, Quer et al., 2017). This can be achieved 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 (Owsley, 1983). The existence of a distinctive SMAS layer at this level is not fully recognized by researchers (Gola 2005, Gola et al. 1994). Indirect SMAS has long been used in plastic surgery techniques, but oro maxillo facial surgery has only recently partially adopted this concept (Meningaud et al., 2006, Stathopoulos et al., 2018). Our study brings quantitative and qualitative evidence of the existence of SMAS at the parotid level.

In the same manner, knowing and understanding subcutaneous layers in related regions of the face, such as the masseteric layer is important in various surgical specialties. The superficial muscular aponeurotic system (SMAS) of the face is a guiding structure for the plastic and oro maxillo facial 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. Also, a systematisation of some of the anatomical conclusions adapted to the new techniques of plastic and repair surgery would equally be of interest, but these would have to take in accont the 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 (Thaller et al., 1990).

A continuous, organized, fibrous mesh, specific to the nasolabial fold and upper lip, frontal, parotid, zygomatic and infraorbital regions, can be distinguished (Ghassemi et al., 2003). Regarding these aspects we seeked to identify the particular morphofunctional features of the SMAS in the meseteric region and attempted to show their practical significance.

Extension of the SMAS arround the midface is significant in the oral region. It is universally accepted that the superficial fascia of different regions of the body 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 has been long disputed. Not long ago (Tessier, 1989) the existence of a morphofunctional cervicofacial system formed by platysma muscles, mimic muscles and superficial cervical and facial fasciae was proposed.

At the facial and anterior cervical regions, the superficial fascia does not play a role of separation between superficial muscles and skin, but a role of cohesion and connection between them.

In our previous studies (Frâncu et al., 2013, Hînganu et al., 2017), we have investigated the disposition of this musculofascial structure in the lateral regions of the face, by dissecting and exploring the fixation means for the soft facial structures to the proximal periosteum. We have identified the ligamentary adhesions, more precisely ligaments and septa that fix SMAS to the bone. These are the main mechanism of antigravitational support for the soft facial tissues. From this point of view, the existence of the SMAS and its particularities plays a crucial role against the phenomenon of "the aging face".

We have also identified and researched the morphology and function of SMAS in the Moebius syndrome (Hînganu 2017). Congenital atresia of the facial nerve leads to important functional deficiencies of the perioral muscles which, over time, affect adjacent regions by means of elongation of their fixation mechanisms and by the appearance of superficial soft tissue prolapse.

Surgical reconstruction techniques which use the temporal muscle tendon transposition are based on the existence of an oral superficial musculoaponeurotic system. In the following paragraphs, I shall approach the oral structures in anatomo-functional terms from a lesser-known perspective: the existence of a unique superficial layer, closely related to both the skin and subjacent muscular layer. Several arguments come in support of this theory, such as:

- it explains the functional mechanisms of the facial expression muscles;
- development of the superficial face and neck muscles and fasciae shows thatthe development ofthe facial expression muscles, superficial and profound adipose tissue, facial nerve and parotid gland are 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 (probably due to the unawarness of its existance), could cause downright mutilating scars to patients.

Functionality of the superficial musculo-aponeurotic system of the face is affected by acquired or congenital lesions of the facial nerve. The lack of motor innervation of the facial expression muscles results in facial kinetic dismorphism and the lack of muscles tone leads to the ptosis of superficial soft tissues.

Posttraumatic injuries can be partially or totally repaired by individualized reconstruction techniques (Kumar, 1990).

Congenital lesions of the facial nerve or of some of its branches have congenital repercussions with an intensity directly proportional to their distribution area.

Moebius syndrome, also called congenital facial paralysis, congenital ocular-facial paralysis or nuclear aplasia is a rare neurological disorder, whose cause has not yet been elucidated. It affects especially facial and oculomotor cranial nerves and its clinical feature is peripheral facial paralysis (Kumar, 1990).

Clinically, it is highlighted from birth, most often by:

- lack of facial expressions, failure of making facial mimics and presence of various types of speech disorders;
- paralysis of the oculomotor nerve, eyelid ptosis, strabismus;
- upon affection of the glossopharyngeal nerve, it may give disturbance in swallowing and chewing and tongue muscle atrophy.

These symptoms may occur by themselves or in various combinations, according to the nerves that are affected, the age and the associated pathology (Carta et al., 2011, Magnifico et al., 2017, Kuhn et al., 1990). The treatment is symptomatic and it aims to improve the patient's quality of life. Thus, logopedic treatment, physiotherapy and home care services can be considered. Surgical cure has specific indications and can provide part of face mobility, on different levels (Terzis and Noah 2002, Terzis and Anesti, 2011, Di Blasio et al., 2014).

Facial paralysis is a major health problem. It is devastating to the patients as it prevents accurate communication and expression of feelings and emotions, thus adversely affecting the quality of life (Mendelson, 1997).

Considering the social and personal impact of facial nerve paralysis, our research goal was to highlight the anatomical and functional changes in the Moebius syndrome, as well as the clinical changes that occur in the face statics and dynamics. We also tried to establish criteria that should be the basis for reparative surgery in this disease. We centralized all the data to develop a map encompassing anatomic and functional changes occurring after long-term lesion evolution.

2.1.2. Scientific context and motivation.

The study of the SMAS derives from the desire to deepen one of the macroscopic anatomical structures that still is the object of controversy.

This topic is a professional challenge as an anatomist. First, the existence of a superficial fascia at the level of the nasal and oral regions remains uncertain, and second, this topic is a subject of interest to many researchers in the field.

The existence of this musculoskeletal apparatus is of major importance in plastic and reconstructive surgery; most of the current surgery techniques regard its morpho-functional features.

Meanwhile, facial nerve reconstruction techniques receive a major help from the SMAS concept, given that the tunneling of the facial nerve branches through the superficial fascia allows the mapping of their distribution.

In oromaxillofacial surgery, the SMAS concept allows the preservation of viable flaps and the maintenance of facial aesthetics after surgery.

Assisting surgical operative teams both in the field of aesthetic and oromaxillofacial surgery on submandibular and parotid glands, has facilitated me to understand the surgical applicability of SMAS.

Also, the existence of SMAS has a particular applicability in the surgical treatment of bilateral congenital athrezia of the facial nerve - *Moebius syndrome*, through temporal muscle tendon transposition. The use of the facial SMAS in the perioral muscular apparatus as an insertion base for the extension of the temporal muscle insertion tendon to this level allows the functional recovery of the speech and nutritional deficiencies of these patients.

As an anatomist, this new concept of facial superficial musculofascial cover is of particular interest because it is the main morpho-functional mechanism that fights against the *„aging face”* phenomenon.

The ligamentary insertions and adhesions of the superficial fascia at the periorbital, zygomatic and temporal level, as well as its continuity with frontal, temporal, parotidian and superficial cervical fascicles allow the antigravitative suspension and fixation of soft cervico-facial tissues.

The SMAS was described in 1974 by Paul Tessier (Leturneau et al., 1988) as an indispensable element of plastic surgery, which was then highlighted by dissections (Mitz and Peyronie1976).

After performing fresh body dissections, topographic histologic and comparative anatomy studies, Thaller et al. (Thaller et al., 1990) reveal 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 performed by tissue plasterization (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, it could not be identified.

The soft tissues of the face are held in a normal anatomical position by a series of fixation ligaments extending deeply to the deep face of the 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 realized.

At the same time, some anatomists are not in consensus regarding the description of this structure some even deny its existence (Jost and Levet 1984, Hollinshead and Rosse 1985, Jacob 1996, Lippert 2000, Berkovitz and Holland 2002, Gosling et al., 2002, Benninghoff-Dreckhahn 2003). These aspects motivated me in choosing this theme for the doctoral thesis and later in the research of this particular anatomical entity.

In the literature, most of the authors agree that SMAS can be macroscopically highlighted at the parotid region, or can otherwise be observed with the microscope. SMAS is continued at the cervical region as the platysma muscle fascia. There is a close match between the development of this fascia and the muscles of the cervical region. This is especially obvious in the adult man, through the presence of a morpho-functional relation between the two layers.

At the head and neck level, the superficial fascia does not have the role of separation between the superficial muscles and the skin of the region, but rather a role of cohesion, thus linking them to the skin (Gardeto et al., 2003).

The implications of SMAS existence are also found in other fields of medicine (see the rate of propagation of an infection from the cervical region to the face).

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

1.	Francu LL, Hinganu D, Hinganu MV. Anatomical evidence regarding sustentaculum facies. Rom J Morphol Embryol 2013; 54(3 Suppl): 757–761.
2.	Hînganu D, Scutariu MM, Hînganu MV. The existence of labial SMAS — Anatomical, imaging and histological study. Ann Anat 2018; 218: 271-275.
3.	Hînganu MV, Stan CI, Ţaranu T, Hînganu D. Morphological changes in support mechanism of superficial face layers in Moebius syndrom. Rom J Morphol Embryol 2017; 58(3): 851-855.
4.	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.
5.	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

2.1.3. Material and methods

2.1.3.1. *Anatomical study*

Our study was conducted on a material consisting of twenty four hemifacials formalized and dissected at the Institute of Anatomy “Ion Iancu” within University of Medicine and Pharmacy (U.M.F.) “Grigore T. Popa” Iasi. The macroscopic study on the dissection specimens was performed using the SOM 62 Kaps operator microscope owned by “Ion Iancu” Institute of Anatomy, U.M.F. “Grigore T. Popa” Iasi. The following overlapping layers on the dissection specimens were identified: 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 regionaltopographic differences.

Dissections were performed layer by layer, starting with preauricular region and ascending toward the internal end of the superciliary arches. On each stage of dissection, mesoscopic images were captured with the 62 Kaps SOM operating microscope. On the dissection specimen, the following superimposed layers were identified, in different topographic locations (frontal lateral, inferior temporal, preparotid, premasseteric, infraorbital): dermoepidermic, subcutaneous fat tissue, superficial fascia, superficial muscular layer, deep fascia, and periosteum and deep musculoglandular items. The conclusive aspects were acquired, examined and further processed to highlight the regional stratigraphic differences.

2.1.3.2. *Surgical study*

Parotid tissue samples have been collected, through a perpendicular incision in the skin, which went deeper until the 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. These patients were clinically and imagistically diagnosed [computed tomography (CT), magnetic resonance imaging (MRI)] with parotid tumors and underwent surgical interventions for total or partial parotidectomy. Surgical interventions have allowed segmental anatomical studies, depending on the surgical procedure. They have provided in vivo visualization of the fascial and muscular structures, and made possible 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).

2.1.3.3. *Histological study*

I have simultaneously conducted microanatomic studies that were made by collecting and interpreting the tissue sample taken from the studied specimens and admitted patients for benign tumor of parotid region and for extended facial lifting procedures. I have collected all the soft tissues of the face, from the skin to the bone plane in the form of small blocks. The fragments collected were processed by paraffin technique and stained with Hematoxylin-Eosin (H&E) and specific staining techniques of the muscular and connective tissue (van Gieson and Szekely).

With regards to the qualitative micro-anatomical study of SMAS within the topographic pattern of the face, I sampled soft tissues from the 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 for a correct examination of the 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). The PRODIT 5.2 program was used for the stereology technique, in order to take quantitative measurements of connective and muscular tissue.

2.1.3.4. *Imagistic study*

Our casuistry also included a group of fifteen patients imagistically explored for esthetic 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. The 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.

Operatory fragments removed from superficial layers to deep fascia have also been used tocompare microanatomical results with CT and MRI aspects.

2.1.3.5. *Clinical study*

The study was conducted on a total of six patients with Moebius syndrome lesions that eighter presented or were admitted in “St. Mary” Emergency Hospital for Children, Iaşi, Romania. The information was obtained from both the observation charts of each patient and from tissue specimens taken intraoperatively (for patients who had such treatment recomendation). Based on these informations, we analyzed changes that occured in our cases, from all clinical, anatomical and histological points of view. We tracked on the studied cases changes in functional anatomy of the superficial layers of the face, which we grouped in terms of clinical manifestationsand correlated with individual pathophysiological substrate.

Clinical observation and palpation was done through specific maneuvers, which highlighted contraction and action of each individual muscle groups and by blocking others. In the cases where a surgical intervention was performed, we collected tissue fragments that have been assessed qualitatively by common staining techniques.

2.1.4. Results

2.1.4.1. *Sustentaculum facies apparatus*

The continuity of SMAS from the occipital region, to galea aponeurosis, frontal region, temporal region to cervical region forms a complex which represents a functional unit that defines the individual faces; the “aging face” is characterized by sagging of the soft tissue due to the loss of elasticity, by subcutaneous and sometimes submuscular fat redistribution, and by loss of the contraction capacity and dragging of the facial expression muscles.

The descending of the forehead can occur when the fixing system of the superciliary superficial structures becomes lax. Dissection at this level shows that the following structures are attached to deep facial skin: the superficial fascicles of the corrugator supercilii muscle, the orbicularis occuli and the procerus between them (Fig. 2.1.1.).

Figure 2.1.1. Insertion of orbicularis occuli muscle and periorbital SMAS.Mesoscopic dissection specimen.

The superciliary arches represent the limit between the face and the forehead; in this area, there is the junction between the superficial fascia of the frontal region and the musculo-aponeurotic system of the face. Additionnaly, there is a temporal ligament (temporofrontal), alongside these muscular transfascial attachments, which is the superior continuation of SMAS at the level of the zygomatic processes.

The dissection continued downward to the junction between the lower borders of the zygomatic arch with the pretragal region, then recessively continued, following the same incision line as in the facelift surgery. The SMAS arrangement in these regions was captured by the operator microscope. We had observed that the anatomical pattern of the ligamentary fixation of the superficial fascia to the facial skeleton defines the boundaries, which divides the face into several regions, and marks the incision lines in surgery. Three of theseare parts of what is seen from the exterior, such as cheek, lateral cheek, pre- and infra-zygomatic parts of the medial cheek, in addition to the other regions: the lower eyelid, the inferior temple, the superior eyelid and the forehead.

The infraorbital SMAS adhesion is a very powerful one, the inferior being limited by the insertion of the great zygomatic muscle and the superior limited by the orbit ledge (Fig. 2.1.2.). It separates the superior regions from the inferior regions of the face. The limit between forehead and face is very distinct at the level of the superficial fascia and it is marked by the SMAS's adhesion orbit aditus, due to the periorbital septum.

Although the SMAS is adherent to the profound fascia of the orbital and frontal region and to the periosteum of frontal bone it continues cranially through these adhesions with the superficial fascia (subgaleal) of the frontal region. Therewith, the lower limit of the face continues with the superficial cervical fascia (superficial fascia of platysma muscle) and form an interconnected anatomical, functional and embryological unit.

The fascia continues both with the temporal part and infraorbitaly, with the fascia of the zygomatic region. The SMAS is adherent to the skin.

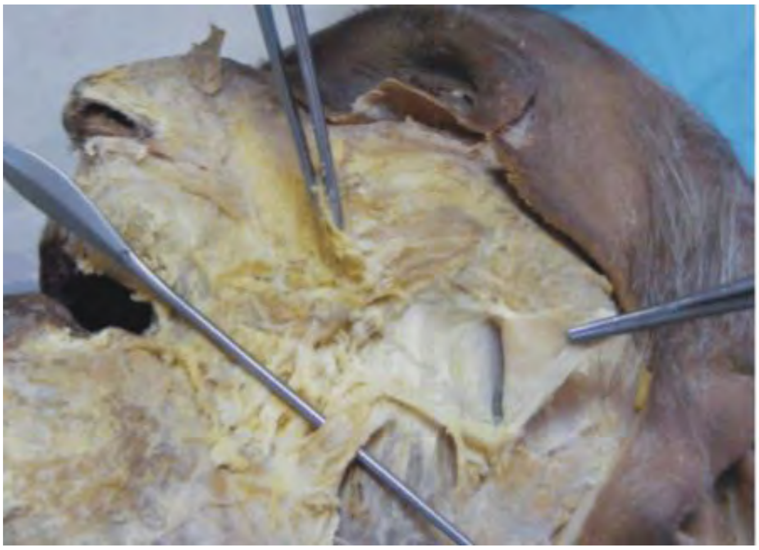

Figure 2.1.2. Limit between superior and inferior region of the face made by great zygomatic muscle.Mesoscopic dissection specimen.

The infraSMAS plane is connected to the profound layers not only by adhesions but also by a cohesion tissue located between the SMAS and the profound fascia. The infraSMAS space is crossed by branches of the temporal, supraorbital and supratrochlear neurovascular bundles (Fig. 2.1.3).

The periorbital adhesions are hard, extensive, robust, inserted directly to the periosteum. Their disinsertion is almost impossible on the dissection specimen. As it has already been shown, all the ligaments in the region including the zygomatic one converge towards each other. Thus, together with the zygomatic ligament, they are the main anti-gravity mechanism of face suspension, however unlike the face, they have no elasticity. They are orientated towards the three axes of the space. Another extremely important inter-muscular link of this region is located on the external angle of the orbit, where it connect the frontalis and orbicularis occuli muscles with the corrugator supercilii (Fig.2.1.4.).

This study shows that the cervicofacial SMAS is a continue fibromuscular lamina located between two fibroadipose laminas, and these three overlapped structures establish different spatial regional relations. In the SMAS's structure, collagen fibers with different dimensions and topographic orientation are found in great amount. The elastic fibers have a lower density, and are even missing in some areas.

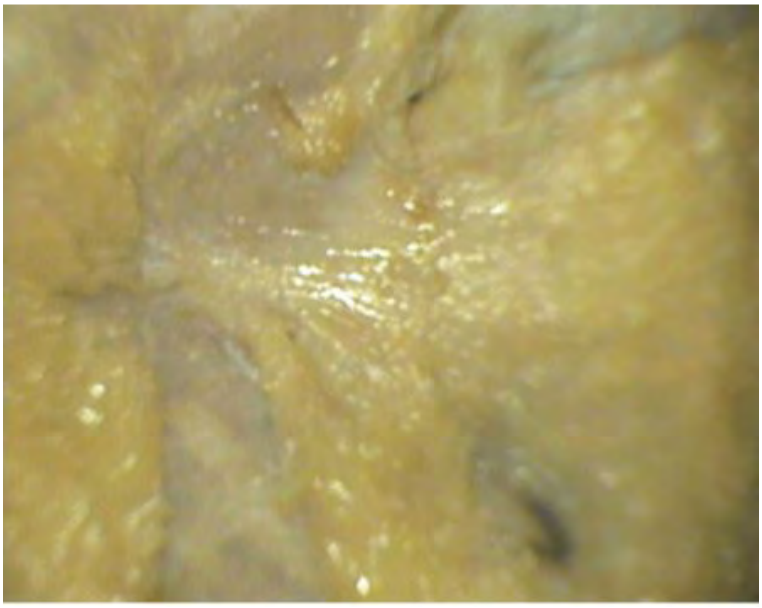

Figure 2.1.3. Convergence of inferior temporal and zygomatic ligaments to the zygomatic process of frontal bone. Mesoscopic dissection specimen.

The collagen fibers are generally arranged in an orderly manner, eighter on longitudinal and transverse planes, or forming a variety of shapes and dimensions, thus losing their individually structure. The latter is most evident in the modiolus.

Figure 2.1.4. Connection septa between orbicularis occuli muscle and dermis which cover large and rectangular adipose cells lobules. Mesoscopic dissection specimen.

The muscular fibers belong to the cutaneous superficial layer, which form the SMAS, and continue with conjunctive fibers (Fig. 2.1.5.). In some areas, they leave the SMAS and cross toward the osteoperiosteum plane.

The inferior border of the temporo-parietal fascia continues with the SMAS from the cheek level, and the anterior border continues with the orbicularis occuli and the frontal muscles. This is why the folding of the temporoparietal fascia during rhytidectomy can increase the tension in the SMAS, orbicularis occuli and frontal muscles. Our microanatomical studies highlighted the reorganization of collagen and elastic fibers in the aging processes, resulting in fiber deformation with subsequent reduced elasticity .

We observed in our specimens the existence of medium sized collagen fibersorderly orientated, particularly longitudinally, as well as the presence of small-sized elastic fibers, concentrated in plots. These represent fixation fascicles, which are separated by large, lipid filled spaces. Infraorbitally, there are connective microseptums between the orbicularis occuli muscle and the dermis (via SMAS) which overlap large, rectangular lobules of fat cells. Our studies revealed the lessening and fragmentation of collagen and elastic fibers within the SMAS structure, consistent with its increase in laxity (Fig.2.1.6.).

2.1.4.2. *Parotid SMAS*

In the parotid region, the superficial fascia presents numerous fat lobules and is part of the SMAS of the head and neck, together with the mimic muscles, blood vessels and nerves that run across. 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.

Frequent highlighting of vertical tracts allowed us to affirm that these structures are a common feature with neighboring facial regions. These vertical tracts allow the removal and reattachment of a SMAS flap. This obviously represents the anatomical base for the exceptional cosmetic results with SMAS based parotidecomy techniques.

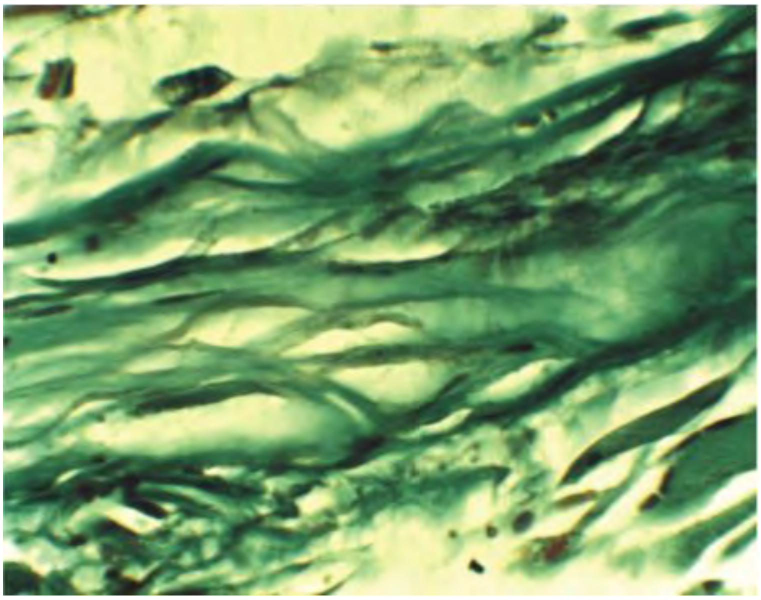

Figure 2.1.5. Powerful fibrous attachments, which cross through the adipose infraSMAS layer (Szekely staining, ×400).

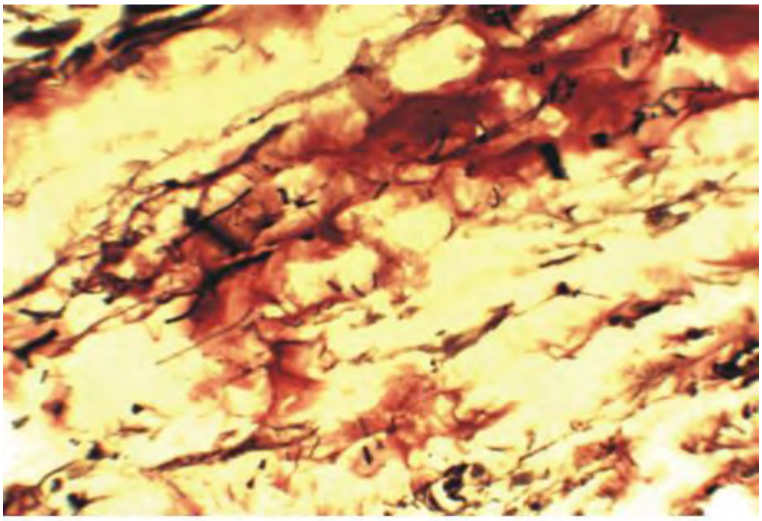

Figure 2.1.6. Reduction of collagen and elastic fibers from SMAS structure at a 70-year-old person (Verhoeff staining, ×400).

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 the skin of the face is done directly through the superficial fascia, an essential constituent of the SMAS (Fig. 2.1.7.). This is true for all the regions of the face, the muscles being an exception. There, blood vessels and terminal branches of the facial nerve cross closely related to the superficial fascia each region of the face, thus offering mechanical protection.

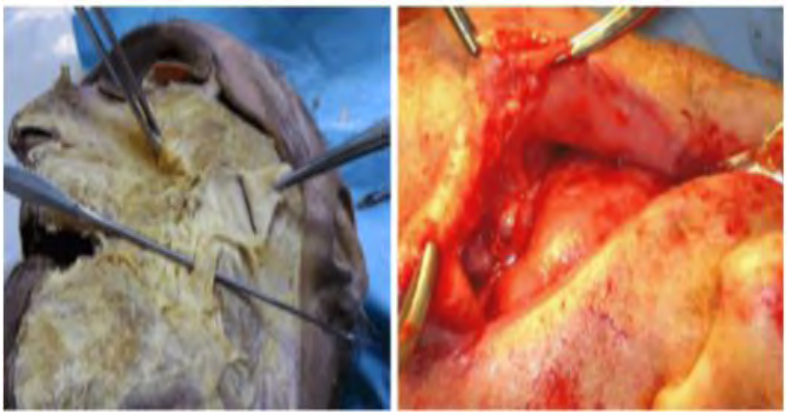

Figure 2.1.7. Removing the superficial muscular aponeurotic system and parotid fascia: dissection specimen (left) vs. intraoperative image (right).

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

Although SMAS is intimately applied to the surface of the parotid gland, a thin, well-defined parotid fascia is identified between the gland and SMAS. Superficial fascia facilitates the adherence of mimic muscles to the dermis and deep fascia (Fig.2.1.8.).

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 (Fig.2.1.9.), 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 oriented obliquely. Elastic fibers are completely absent.

Figure 2.1.8. SMAS adhesions to deep fascia that secures the side of the parotid region. Dissection specimen.

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

These observations support the quantitative data obtained on CT images from the group of patients that underwent surgery. In the 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. In the parotid region, the superficial layers have different thicknesses: the supraSMAS fibrous tissue is well represented, the SMAS is a structured, dense organized connective tissue, the infra SMAS fat tissue forms a very thin lamina under which lies deep to the parotid fascia.

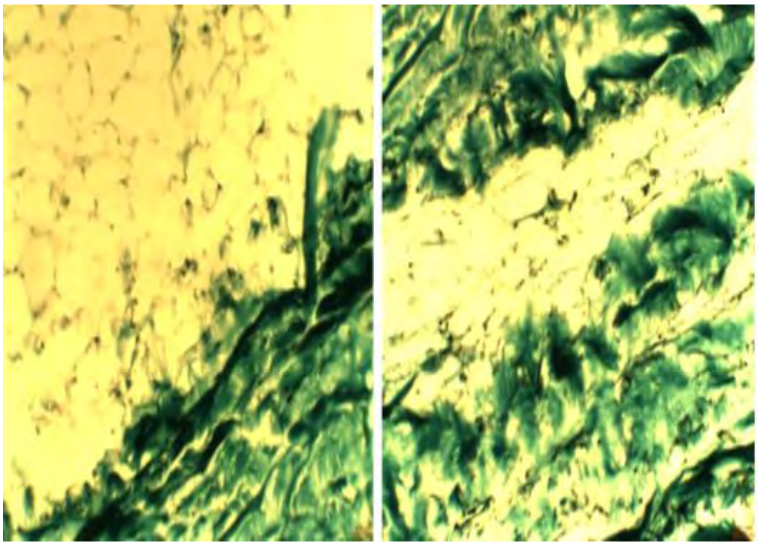

Figure 2.1.9. Fibrous-adipose supraSMAS (left image) and infraSMAS (right image) tissue is well represented in the parotid region. Szekely staining, ×200.

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 the branches of the facial nerve are running (Fig.2.1.10.).

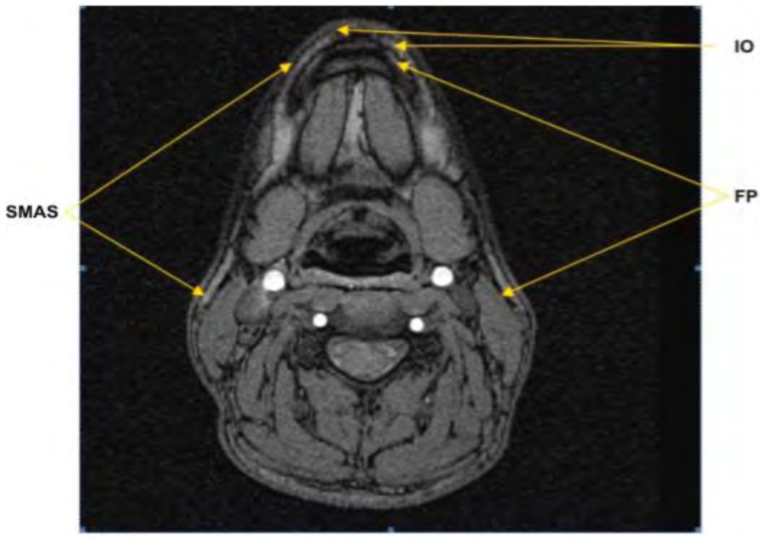

Figure 2.1.10. Horizontal section through the lower edge of the mandible; SMAS and deep fascia (FP) between the parotid and mental region; transSMAS insert of the orbicular mouth, inferior fascicle (IO). SMAS: Superficial muscular aponeurotic system.

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).

2.1.4.3. *Masseteric SMAS*

SMAS can be seen macroscopically and microscopically in the masseteric region. Laterally, it 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.

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

The neurovascular elements in its immediate neighborhood are numerous and complex. One must emphasize the complexity of the parotid region where the facial nerve and the branches of the external carotid artery are found. The 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 massteric 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 (Fig. 2.1.11.). 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 relations of the superficial masseteric fascia.

The masseteric region makes the transition to the zygomatic and temporal regions. The morphological, macro and microscopic differences between these regions are very concise. Superficial thick masseteric fascia continues anterior and superior with the zygomatic fascia, and posterior and superior with the temporal one. Inferior and posterior, at the level of the parotid gland, the superficial fascia gives rise to the anterior capsule of the gland.

Anterior and medially, it is continued by the superficial fascia of the cheek and it forms fibrous tunnels through which branches of the facial nerve are passing (Fig.2.1.12.).

Figure 2.1.11. Branches of facial nerve related to SMAS. Dissection specimen. SMAS: Superficial muscular aponeurotic system.

Superficial masseteric fascia is continued downwards by superficial parotid and cervical fasciae, where it originates from. The most important features of soft tissues stratigraphy at this level are:

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

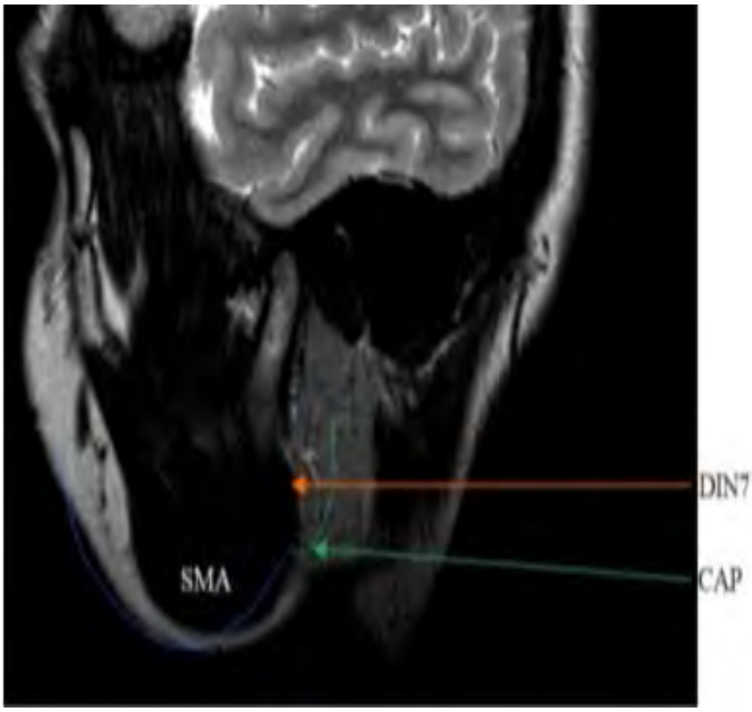

Figure 2.1.12. Anterior parotid capsule (CAP); intraparotid division of the facial nerve (DIN7).

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

- subcutaneous fat;
- superficial fascia;
- the branches of the facial nerve;
- deep, masseteric fascia;
- the muscular layer, represented by the masseter muscle.

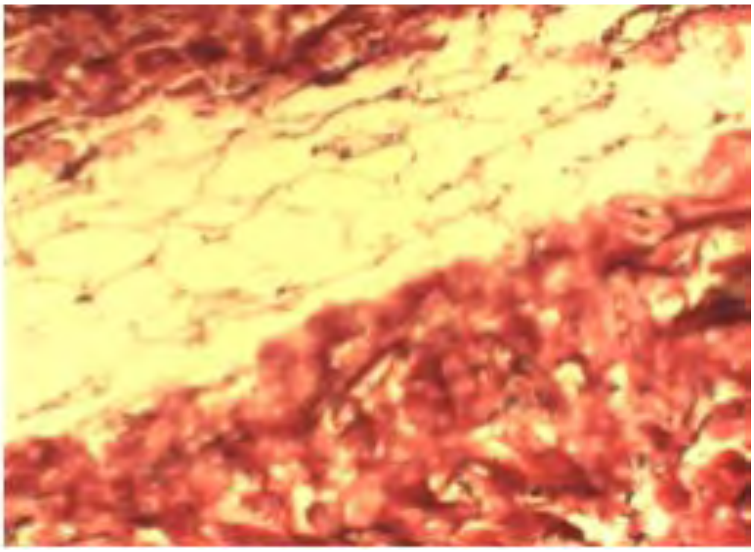

Figure 2.1.13. Succession of superficial layers in the parotid region, top-down: SMAS, adipose tissue infra-SMAS and parotid fascia. Verhoeff's staining, ×400. SMAS: Superficial muscular aponeurotic system.

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

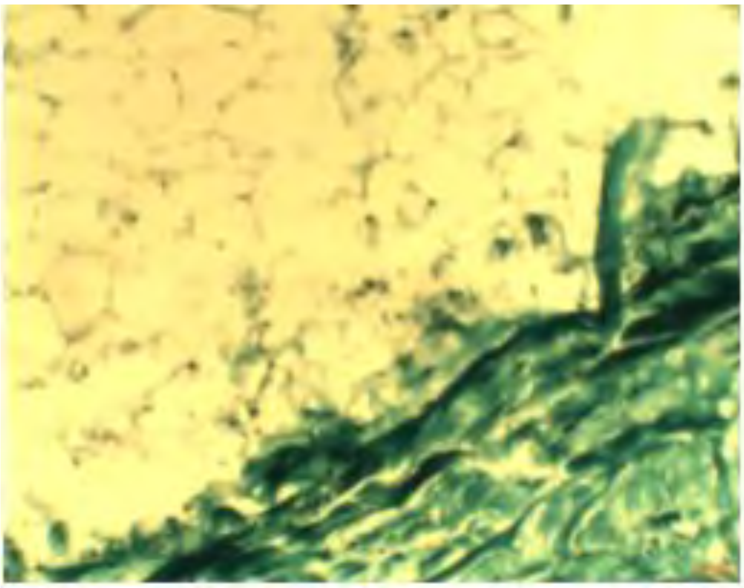

Figure 2.1.14. SupraSMAS fibro-adipose tissue, well-represented, in the parotid region. Szekely staining, ×200. SMAS: Superficial muscular aponeurotic system.

The distance to the deep fascia is increased, the infraSMAS fat layer being well represented with oblique orientation; the elastic fibers are completely missing. The superficial 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 to the arrangement in the parotid region.

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 (Fig. 2.1.13, 2.1.14.).

2.1.4.4. *Labial SMAS*

As main feature, the oral region presents an infraSMAS space filled with adipose tissue. This space provides a route of spreading for an infection into neighboring regions.

Macroscopic study results show that the infraSMAS connective tissue from this level contains fibers organized as conjunctive tracts which separate the fat lobules. Its structure also includes muscle fibers 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~~to~~ the direction of the movements of the masticatory muscles (Fig. 2.1.15.).

Starting from the zygomatic arch and progressing towards the mandible, SMAS and infraSMAS tissue are shaping the appearance of the cheeck by the amount and shape of the adipose tissue, which they delimit. Here, it also provides skin firmness at the level of the deep planes. Oral muscles adhere intimately to the deep surface of the skin, crossing the superficial fascia. At this level, in the thickness of the musculoaponeurotic system adipose tissue is trabeculated by conjunctive septa and labial vascular nervous structures. The insertion is firm, almost impossible to dissect, making classical anatomical dissection (macroscopic) of the superficial fascia extremely difficult.

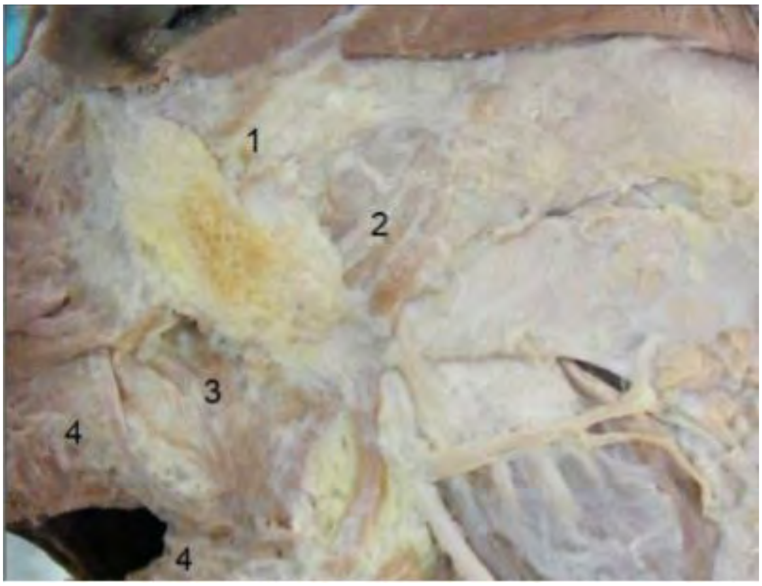

Figure 2.1.15. Muscles of facial expressions at the mouth angle: 1 — zygomaticus minor, 2 — zygomaticus major, 3 — levator anguli oris, 4 orbicularis oris. Dissection specimen (SOM 62 KAPS microscope, ×20 oculars), 3/1 scale.

Thus, this region is best approched with the operator microscope. 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. We have to emphasize once again the importance of the zygomatic ligament, which also contributes to the raising of 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, the vector direction of the traction forces acting upon them), macroscopically visible.

It has two portions: medial (periorbital) and lateral (tragally) (Fig. 2.1.16.). Dissecting downward to the lower lip, we have easily revealed a musculo-conjunctival infradermic layer on anterior mandibular surface, which offers attachment for mental muscles.

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.

Histological uses of traditional staining specific to connective and muscular tissue reveals that the 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. In regions where the muscular fascicles of orbicularis oris muscle interconnect, we find collagen fibers with the same longitudinal disposal, out of which thin paths descend to the skin (Fig. 2.1.17.).

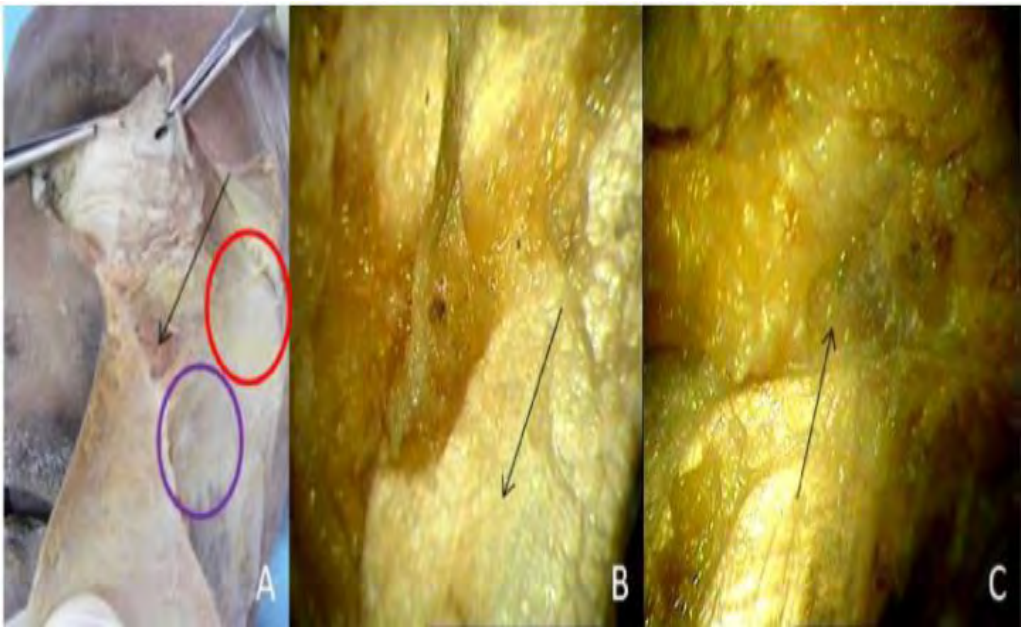

Figure 2.1.16. Image A shows both parts of the zygomatic ligament within the two circles. The first part, marked by the red circle, represents the tragal part, magnified in picture B. The purple circle represents the medial part which adheres on the skin of the cheek, magnified in picture C. The arrow points out the direction of ligamentary fibers, from the zygomatic arch to the cheek in all three pictures. Dissection specimen (SOM 62 KAPS microscope, ×20 oculars), 20/1 scale in B and C images.

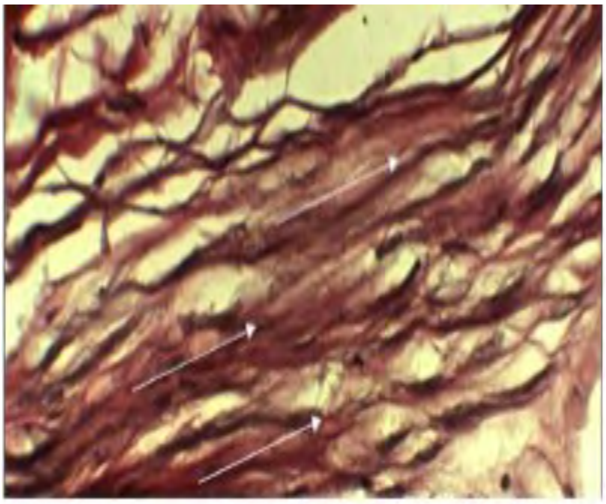

Figure 2.1.17. SMAS medially to nasolabial sulcus with longitudinal disposal of collagen fibers, marked by white arrows. Col. Verhoeff, ob. 40×. twined with fascicles of muscular fibers, while the elastic fibers are almost absent.

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 the lips, both macroscopically and microscopically.

Verhoeff staining shows lack of elastic fibers and prevailing of the interstitial tissue.

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

- medially, it gives insertion to the orbicularis oris muscle (inferior fascicle) and then to the depressor anguli oris muscle (Fig. 2.1.18.);
- laterally, it becomes mobile, ascending first above the jugal fat pad, the buccinator and then the masseter muscle.

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

Even if superficial fascia gets progressively thinner towards the modiolus, its thickness is still enough to appear as a clear tissue strip on MRI. The same is true for dissected specimens. Ascending to the nasal septum base, the two fasciae (superficial and profound) are united into a dense conjunctive structure.

The more we approach the nasolabial groove in its medial part, the clearer the superficial fascia becomes. It provides protection for both the superior branch of the angular artery and for the superior labial fascicles of the buccal branch from the facial nerve. Injuries of these branches of the facial nerve or of its trunk cause static deformities of this region, alimentation and phonetic disorders, depended on the scale of the injury.

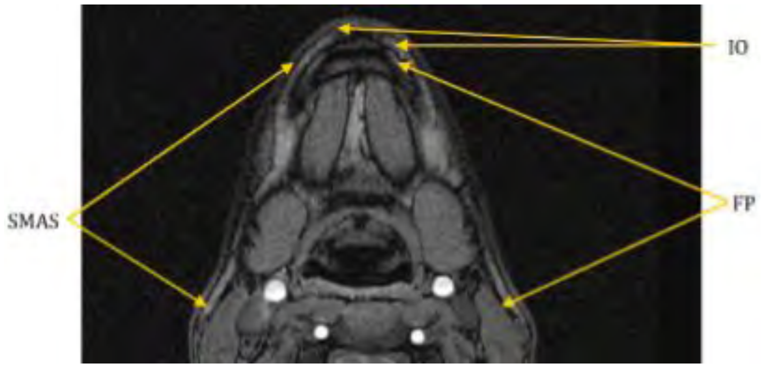

Figure 2.1.18. MRI horizontal section through inferior mandibular margin; SMAS and profound fascia (PF) between parotidian and mental region; TransSMAS insertion of orbicularis oris, inferior fascicle (IO).

These disorders are reflected in the skin insertion ofthe muscles and the continuity of SMAS toward the other regions that involve their step by step transmission (Fig. 2.1.19.).

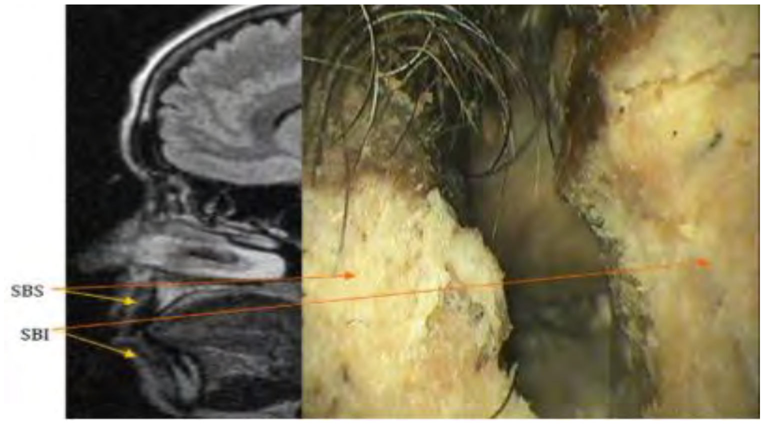

Fig. 2.1.19.SMAS at upper lip (SBS) and lower lip (SBI).

2.1.4.5. *Congenital facial atrezia*

The clinical examination and functional exploration of the first patient showed the following aspects related to atresia of the facial nerve: asymmetric facies, lagophthalmos, impossible closure of the eyes and mouth, decreased lacrimation, loss of taste.

Besides these aspects, the patient experienced symptoms related to damage of other cranial nerves, such as: abducens nerve – impossible lateral gaze, convergent strabismus, photophobia, myopic astigmatism; hypoglossal nerve – difficulty in chewing, preferring soft food, tongue protrusion, difficulty in swallowing. Cranial nerve injuries are associated with other congenital malformations: microcephaly, tear duct changes, hypoplasia of the wing nose, hooked nose, short filter.

In another patient, we observed clinical signs given by facial nerve damage, with craniofacial dysmorphia, eyelids opening upward and laterally. Lesion of the oculomotor nerve determined convergent strabismus, disorders in eyeball motility, with the absence of abduction and convergence of the eye, obliquity of palpebral fissureand divergent deviation.

All of these were correlated with congenital atrophy of the optic nerve (blindness), impossible closure of mouth, micrognatism, posterior rotated ears, triangular facies, psychomotor retardation.

The third patient also presented symptoms associated with damage of the facial nerve, facial dysmorphia with myopathic facies and ptosis of the eyelid more precisely, but also by with injuries associated with lesions of other cranial nerves – paresis of oculomotor nerve, bilateral paralysis of the abducens nerve with strabismus and bilateral palpebral ptosis; hypoglossal nerve injuries – swallowing disorders.

All these were associated with circular facies, hypertelorism, mongoloid eyelid slits, facial hypoplasia, broad bridge of the nose, low-set ears, pectus excavatum, prominent nose base, ears dysplasia, weak voice, intellectual disability, cognitive and psychomotor delay.

Another patient had clinical signs of facial nerve injury at the face level: facial dysmorphia, inexpressive facies, narrow slits eyelid, associated with small nose, small mouth, lowered commissures, micrognatism, ears dysplasia both being small and round, discrete right hemiparesis and moderate delay in psychomotor development.

The fifth patient had the following changes of the face: inexpressive facies, short eyelid slots – given by bilateral facial nerve injury. He had specific clinical signs given by oculomotor nerve damage: limited movements of the eyeballs associated with prominent upper lip, evident micrognatism.

The last patient had the following symptoms: bilateral facial paresis (inexpressive facies), cranial nerve paresis (ocular mobility disturbance) associated with small, asymmetrical tongue, significant phonation disorders, dental malposition. Neurologically, this patient had medium/severe delay in mental and language development (intelligence quotient – IQ = 35–40).

After studying these patients, we found that clinical symptoms were directly correlated with the level of cranial nerve injuries. Unilateral lesion of the facial nerve causes facial dysmorphism by ptosis of the superficial tissues on the same side and by flattening wrinkles expression. In cases where the damage occurs bilaterally, the patient's face is like an inert mask. In both cases, the evolution in time of symptoms leads to dysfunction of the facial muscles, especially the muscles of the jaw, controlled by the trigeminal nerve.

We found that the most exposed areas of the face to present dysmorphism are the central ones: oral, nasal, orbital. On the lateral regions of the face, where superficial fascial has insertions on the bone, there are two forces that act on these attachments: those caused by facial muscle contraction (engaged in a whole as SMAS) and those caused by masticatory muscles.

Clinical signs of the damage in this lateral region appear in time, after a mean duration of five years from the onset of central facial dysmorphism.

Affection of the infraorbital, zygomatic and jugal regions is a consequence of the appearance of laxity in the fixation structures of SMAS, combined with changing vector forces acting in these regions, due to gradual installation of central facial dysmorphism and "fatigue of masticatory muscle". The main defect that occurs in this medial region is quantitative loss of adipose tissue (the reduction of Bichat's fat pad).

Moebius syndrome affects both the sequentiality and the clinical manifestation of these three regions. Initially, malfunctions occur in the medial regions, especially in those with occlusive functional characters. Impairment of the medial transition regions installs secondly, substantially due to secondary functional lesions in the medial regions. Finally, clinical changes in the lateral regions occur latest, due to ongoing straining manifested for long time.

The pathophysiological mechanism of these clinical manifestations is quite similar to the phenomenon of “aging face” and consists in loss of the quality of various mechanisms of anti-gravity fixation of superficial cervical and facial structures. These phenomena have resulted in facial soft tissue ptosis that further affects the functionality of other facial regions.

Emergence of these clinical manifestations represents criteria for surgical treatment, which is the only treatment option capable of yelding mechanical and anti-gravitational support of the structures of the SMAS, achieved though cutting of the original, incompetent means of fixing (rejuvenation) and transfer of the temporal muscle function to the mimic muscles (orbicularis oris).

Common stainings revealed the atrophied muscle fibers correlating with the appearance of excess fibrous connective tissue, especially in the medial, relational region of the face (Fig. 2.1.20.).

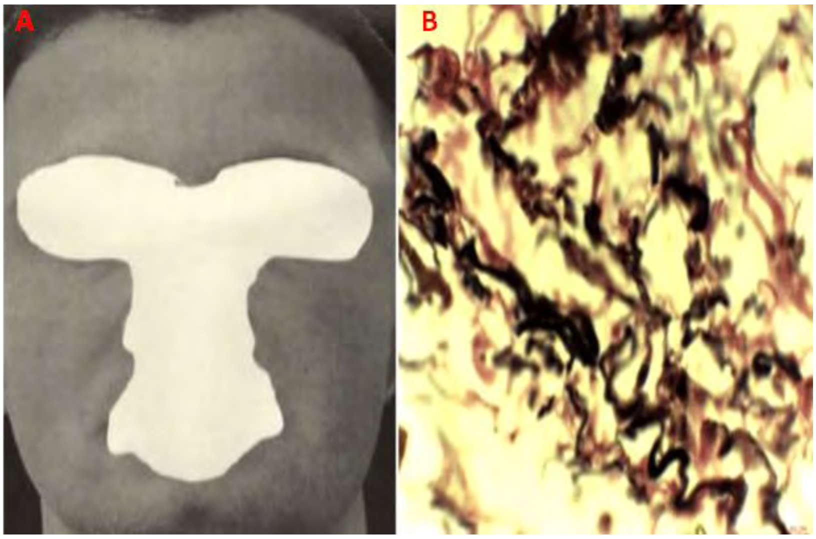

Figure 2.1.20. A=median region of the face; B=numerous elastic and collagen fibers, medium size and muscular fibers underrepresented in the structure of SMAS from the jugal region. Verhoeff staining, ×600.

Laxity of fixation elements can be identified at the level of the lateral regions of the face (Fig. 2.1.21.), through the proximity of the periosteum, by collagen fiber elongation.

In the medial passage region (Fig.2.1.22.), the changes appear most probably at a later stage. It is the last region of the face that suffered degenerative changes following the loss of tone and contractility of the mimic muscles. The nasolabial groove, in particular, remains last redoubt. At its level, we have not detected any significant changes in advanced stage morphology of the disease in the studied patients.

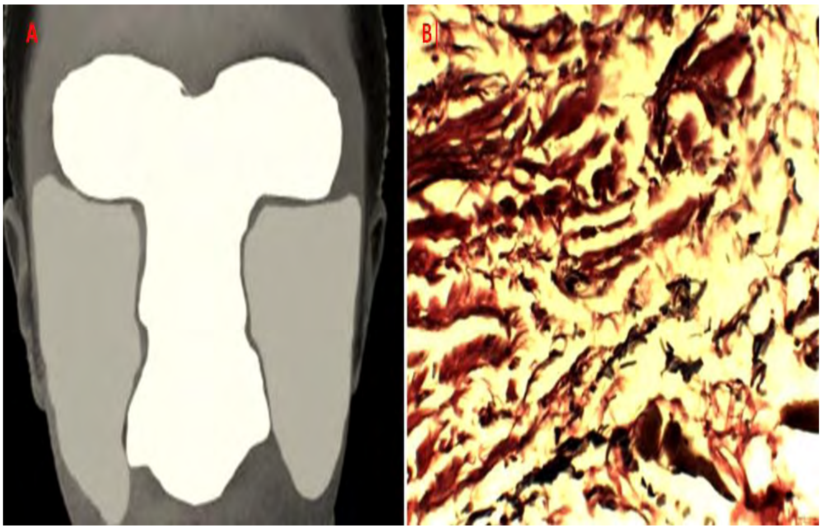

Figure 2.1.21. A=medial regions of the face; B=collagen and elastic fibers from SMAS in lateral part of nasolabial groove.Verhoeff staining, ×400.

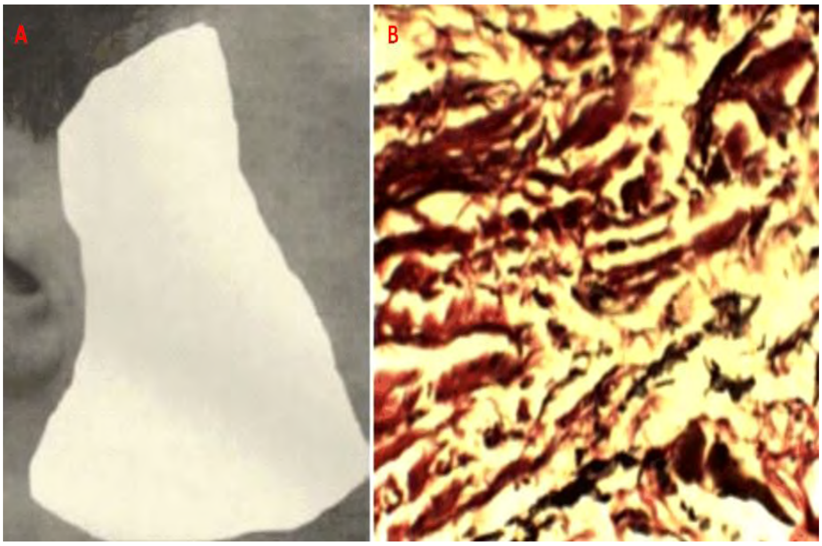

Figure 2.1.22. A=lateral regions of the face; B=periorbital SMAS, with dense collagen fibers, medium and abnormally long, arranged longitudinally. Van Gieson staining, ×600.

2.1.5. Discussions

The soft tissue architecture of the face can be described as being arranged in a series of concentric layers: skin, subcutaneous fat tissue, superficial fascia, facial expression muscles, deep fascia (parotidomasseteric), facial nerve plane, parotid duct plane and oral fat (Stuzin et al., 1992). The anatomical relationships between these layers are:

1. *Superficial fascia* covers the superficial muscles of facial expressions (platysma, orbicularis oculi, major and minor zygomatic);
2. *Deep facial fascia* represents a continuation to the face of the cervical fascia; its importance lies in the fact that the branches of the facial nerve are situated profound to this fascial layer;

3. There are two types of relationships between superficial and deep fascia: in some regions, the fascial planes are separated by an areolar plane, in other regions, the two fasciae are intimately adhered to each other by a series of dense fibrous attachments.

Also, there are two distinct types of SMAS, differentiated by the presence or absence of fat lobules separated by conjunctival tracts (Dzubow 1986).

The term SMAS introduced in 1974 defines a musculofascial sheath that continues the frontal muscle to the platysma and is "an amplifier of the facial muscle contraction". 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:

- SMAS transmits the contraction of the muscles along the parallel network to the skin plane
- SMAS transmits the resulting effect in a perpendicular direction to the skin through fibrous extensions from SMAS to dermis.

In addition, soft tissue plans are maintained in a normal anatomical position by a series of restraining ligaments coming in from the depth, fixing the facial structures to the suprajacent dermis.

We will insist on the regional particularities of SMAS, presenting data allowing *anatomical conclusions adapted to new rejuvenating surgical techniques*.

Examination of the anatomical specimens revealed general aspects that correspond to the classical data, as well as particularities regarding specific features differentiated by topographic areas.The controlled dissection of the face reveals the structure of each region, in concentric layer. SMAS occurs in two distinct forms, whether or not associated with the presence of lobular adipose tissue traversed by connective tracts. Generally, the posterior part of the SMAS is fixed and the anterior one is mobile.

2.1.5.1. P***eriorbital SMAS - Sustentaculum facies apparatus***

At the **periorbital region**, SMAS exists as a well-defined entity recognized by researchers (Yousif 1995, Marten 1997). The superficial fascia of the region and, implicitly, SMAS, has several features that differentiate it from other regions because it is a *craniofacial junction area*. The passage from one head compartment to the other (from the face to the forehead) is located at the supraorbital level. A similar compartment will be found *cervicofacially*, another junctional area: in the submandibular region, it represents the transition from the face to the cervical region. The boundary between forehead and face is evident at the superficial fascia and it is marked by SMAS adherence of the orbit by the periorbital septum (Rohrich and Pessa 2007).

As our anatomical studies demonstrated, in the periorbital region, the adipose supraSMAS and the infraSMAS layers are crossed by conjunctive fibers fascicles (microseptum) more or less obliquely arranged, which come from the superficial and profound surfaces of the SMAS. The infraSMAS fibrous connecting tissue precisely compartmentalizes the face fat and acts like a barrier, which restrains the progressive movements of the superficial planes.

There has been recently demonstrated that these compartments gain and loose in weight in different proportions (Rohrich and Pessa 2007). In time, the gravity tends to weaken the ligament support and to initiate the progressive tissue migration (Yousif 1995, Marten 1997), with the changing of the spatial face tissue relations. The rate of face aging partially depends on the face's fat modifications in time (Dallara, 2009).

The SMAS exists in the periorbital region as a very well defined entity and it is acknowledged by researchers. Periorbital SMAS, have some characteristics which individualize them from other regions by of the presence of the craniofacial junction, which is reflected in the profoundness by a cushioned craniofacial junction area, specifically human (Gassemi et al., 2003, Coleman and Grover 2006).

A similar compartment in the cervicofacial junction area, in the submandibular region, represents a limit that is continued in profoundness by the existence of the buccal floor. It was signaled that there fixing and tense areas of the SMAS, such as the medial side of the mandibular branch or of the orbit (Ghavami et al., 2008) assessing their importance is limited. The stabilizing effect that occurs in these ligamentary connections ensures the movement that results from the muscular contraction of each zone, so that, at least in youth, the movement is not transmitted to the superficial fascia of the adjacent areas (Mendelson, 1997).

The ligamentary fixation has a shock-absorbing effect, which modulates the degree of sliding during muscle contraction. At this level, the SMAS has very powerful fixation means, embodied by the supraorbital ligament adhesion and by the nearby superior and inferior temporal septum. Weak points can be present in some topographic regions, and appear mainly during aging.

Periorbitally, such areas are only in the inferior and the lateral parts of the region, where the adhesions and the cutaneous muscular attachments are weaker and less dense and where progressive decreasing of the fibroadipose infraSMAS layer and the tissue around the eyes is moving inferiorly.

In the superior part, the dragging of the frontal tissues is orientated to the resistance point of the supraciliary arch, involving the movement of the eyebrows, which becomes more protruding. Following the weakening of the periorbital tissue and the change of spatial distribution of the fibroadipose layer (Coleman 2004, Gosain et al., 2005), the arches and the convexities disappear and give a rounded aspect to young faces. The structural related differences give particular abilities in the emotional face expressions, but also have different capacities of maintaining the anatomical substrate of the face and ensure flaps nutrition in the facial lifting (Hamra, 1996).

The periorbital SMAS has very strong means of fixation represented by the supraorbital ligament adhesion, but also by the upper and lower temporal septae that are in the immediate vicinity. In surgery, these ligaments and septae mark the dissection lines of the region.

In the center of the region there is the orbicularis oculi muscle, deeply located. Of its three portions (lateral, medial, and palpebral), the middle (palpebral) is adherent to the superficial fascia via the connective tissue mentioned above. This causes skin movements to be determned by mobilization of the muscle. The palpebral part of orbicularis oculi muscle forms together with SMAS and adjacent tegument the eyelid (both upper and lower). Thus, SMAS actively intervenes in the sphincter action of the muscle, protecting the eyeball.

The orbicularis oculi muscle, in its upper portion, may frequently present *interconnections with the lower fascicles of the epicranian digastric (the frontal muscle). These orbiculo-frontal interconnections are not constant and can differ quantitatively and qualitatively in the same person, on the left or right. This possible difference explains the asymmetric aspect of the face in some patients. When it exist, it explain the simultaneous movement of the upper eyelid and forehead dermis in both directions (gravitational, caudal or cranial).*

Another extremely important intermuscular relation in this region is given by the external angle of the orbit between the frontal muscles and the orbicularis oculi on the one hand and the corrugator supercilii mucle on the other hand.

The main vasculo-nervous bundle at this level is located in a splitting of the superficial fascia, being relatively difficult to dissect. Its location is between the zygomatic ligament and the periorbital adhesions.

Regarding the relationship between SMAS septae and ligaments on one hand and branches of the above-mentioned neurovascular bundles on the other it can be said that these connective fibers are specific markers of the respective nerves and blood vessels and are included in the connective tissues portnerv and portvas. Thus, the branches of the supraorbital nerve pass along the periorbital septum to reach under the galea aponeurotica.

Supraorbital ligament adhesion is condensed along these nerves, providing them mechanical protection. In their trajectory, the infraorbital nerve branches follow the subSMAS margin of the inferior periorbital septum starting from the lower eyelid.

The only exception is the supratrochleary nerve, that crosses the plane between the ends of the corrugator muscle. The anterior branch of the zygomatic-temporal nerve is in relation to the lateral orbital ligament. After its emergence from the zygoma, the zygomatic-facial nerve, is associated with the inferolateral portion of the periorbital septum. The zygomatic-temporal and supraorbital cutaneous nerves perforate the superficial fascia.

2.1.5.2. *SMAS in temporal region*

In the ***temporal region****, the superficial fascia is part of the same musculofascial complex that lies between the galea aponeurosis and platysma muscle. It has the same morphological features with the anatomical entity that we describe as SMAS and is therefore part of it. This is also demonstrated by the common embryological origin of this superficial fascia and the mimics muscles. The only difference between the superficial fascia of the temporal region and the rest of the SMAS is that it does not have attachments for mimics muscles because they do not reach the fascia.*

In the temporal region, three fascial layers are distinguished: the temporoparietal fascia, superficial lamina of the deep temporal fascia and the profound lamina of the deep temporal fascia.

Similarities include the existence of the same fixation means and its continuity with SMAS in neighboring regions (periorbital, zygomatic, frontal, and supraauricular).

The adhesion elements of the region consist of:

- temporal ligamentary adhesion that separates it from the galea aponeurosis
- superior and inferior temporal septae
- periorbital septum separating it from the periorbital region

- zigomatic and maseterin ligaments that separate it from their homonymous regions(Gola 2005).

Noteworthy is that fibro-adipose adhesions between SMAS, deep fascia and periostum limit mobility and also maintain the firmness and gravitational independence of superficial layers. They are the only ones of this type present in the above region. Thus SMAS supporting elements play the most important role in delaying the phenomenon known as the aging face. This consists in wrinkle appearance when supporting elements become lax as the dermis moves in gravitational sense (Hamra, 1996).

Because of this, the temporal region is the place of choice for performing facial lifting that uses the SMAS to tension these adhesions, septae and ligaments. This is clearly demonstrated by theefficiency of the surgical maneuver, especially after cutting the temporal ligaments.

The main tissue plane from depth to surfacetissues include the deep fascia/ pericranium, subSMAS plane, SMAS/galea aponeurotica, subcutaneous tissue and skin. One can observe the form of the ligamentspassing through the subSMAS plane to the superficial tissue. These are classified true ligaments, septae and adhesions. Temporal ligament adhesion (ALT) is located at the intersection of temporal, frontal and periorbital regions. From this level, there will be 3 branches with radial disposition towards the three angles: superior and inferior temporal septae and supraorbital adhesion.

Temporal ligament comes from the frontal bone periosteum and extends to the anterior end of the upper temporal septum. It is inserted into the superficial fascia at its junction with the galea aponeurosis, on the deep surface of the frontal muscle.

The superior temporal septum (STS) comes from the superior temporal line and inserts at the junction line between the superior temporal fascia and the galea aponeurosis ga. The inferior temporal septum (STI) comes from the lateral end of the temporal ligament and is inserted into the external auditory meatus.

The STI splits the region into a superior and inferior compartment. The superior compartment can be dissected safely, as it does not contain importat vascular and nervous branches. The inferior one contains temporal branches of the facial nerve. The presence of a sentinel vein immediately below the inferior temporal septum is of importance , as it is accompanied both above and below by the branches of the temporal nerve.

Under the inferior temporal septum there is a triangular space in which there are adipocytes and numerous fibrous adhesions. This adipose layer hosts numerous blood vessels and cutaneous fibers. The respective fibrous adhesions will form inferiorly the z*ygomatic ligament* by condensation.

Specific to the temporal region is the presence of fat lobules. The fact that they are adherent to the superficial fascia, but not to the deep one raises special challenges related to their preservation in surgeries, asthey are crossed by numerous vascular formations (including the superficial temporal vein), whose presence at this level guarantees the normal revascularization of the region.

The frontal branch of the facial nerve runs parallel to the frontal branch of the superficial temporal artery, deep in relation to the temporoparietal fascia. For this reason, classical subfascial dissection to protect the nerve does not have anatomical substrate. Close subcutaneous dissection, immediately beneath the hair follicles, is recommended to avoid nerve damage and excellent exposure of the temporoparietal fascia for rhhipitectomy, with the protection of the auriculotemporal nerve and superficial temporal vessels (Tellioglu et al., 2000).

Sometimes a very thin muscle layer was observed on the outer surface of the temporoparietal fascia below the temporal line.

Unlike the other superficial fasciae, the one from the face ~~it~~ comes in contact with mimic muscles, and in some places adheres to it.

Its fibrous extensions go back to the bones of the proximity but above the cutaneous insertion area. They also delimit subSMAS spaces or visceral lodges as we will see in the parotid region.

The nerve branches accompanying these extensions makes them of particular importance as trace markers.

At the level of both regions, the complex formed by the superficial fascia and the adjacent muscles act, as means of suspending of the facial dermis. It keeps the region's skin tension, prevents it from descending, and through the muscles produces facial expression. Like the peritoneum,the superficial facial fascia separates the superficial layers from the deep ones, fixes the skin to the periosteum and presents condensations on the directions of the neurovascular bundles.

2.1.5.3. ***Nasal SMAS***

At the level of the nasal region, two distinct areas both morphologically and functionally should be discussed: nasolabial groove and SMAS on the wings of the nose. At the level of the nasolabial groove, the superficial fascia of the nose adheres to that of the cheek, thus forming a thickening that adheres to the periosteum. Laterally to the groove, there are fibrous extensions of the superficial fascia that cover tall fat lobules.

The deep muscular layer of the nasolabial groove is formed by the buccinator muscle. At the nose wing, this layer disposal is no longer preserved. Under the dermis, we find collagen fibers, adipose cells and fibers mixed with nasal muscle fibers. Together, these elements form a layer between the dermis and the muscles of the nose.

The Skin insertion of zygomatic muscles, levator labii superioris and levator anguli oris muscles are found medially to the nasolabial groove. In addition, the orbicular oris muscle adheres intimately to the deep face of the skin.

The nasolabial groove is the result of the dermal insertion of the zygomatic muscles, levator labii superioris and levator anguli oris muscles.

Other authors (Letourneau et al., 1988) consider that at the level of the nasal region there are five layers which, from superficial to deep, are: subcutaneous adipose tissue, fibromuscular layer, profound adipose layer, a longitudinal fibrous layer, and a layer containing the interdomal ligament. They believe that at this level SMAS is the second layer, the fibromuscular one, which is interconnected with the allar muscles and distributes their force to the dermis.

We can conclude that the role of SMAS in the nose is to interfere with breathing along with the allar muscles.

2.1.5.4. *SMAS at the genian region*

The **genian region** can be divided into *5 aesthetic subunits*: buccal, infraorbital, zygomatic, parotidomasteric and supraauricular. The genian region extends between the zigomatic arcade and the infraorbital portion of the orbicularis oculi muscle, superiorly and the cervical edge of the mandible inferiorly (Letourneau et al., 1988).

Medially it extends to the nasolabial groove, and the lateral boundary follows a line between the gonion and the pretragal area. From a surgical point of view this region can be subdivided into a medial and lateral portion. At this level, SMAS has the same morphological and functional features regardless of the subregions we refer to. The superficial fascia facilitates adhesion of the mimic muscles of the region to the dermis. This reveals that the oral superficial fascia will follow the masticatory movements of the buccinator and masseter muscles, but also those of the major and minor zigomatic muscles. These muscles are the subSMAS cover and thus take part in the formation of a unitary complex together with the superficial fascia.

SMAS is fixed at the top of the region through the inferior part of periorbital septum and at the zygomatic arch. Medially it adheres to the maxillar periosteum through the fibrous condensation of the nasolabial groove. Inferiorly it is inserted at the lower edge of the mandible from where it continues with the superficial fascia of the platysma muscle. Laterally it forms the anterior sheet of the parotid fascia. The major zigomatic muscle adheres through its anterior surface to the superficial fascia. Because of this, SMAS elevation is concomitant with the muscle contraction. Although SMAS is intimately applied to the superficial surface of the parotide, a thin but distinct parotid fastia fascia is identified between the gland and SMAS.

2.1.5.5. *SMAS in parotid region*

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 et al., 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 et al., 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 et al., 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 and the infraSMAS adipose tissue forms a very thin blade under which there is a deep parotid fascia.

The latter has very dense structure, its collagen fibers constituting a dense structure with homogeneous masses in which the separated fibers are not individualized. We can affirm they have a lamellar disposal (Gola et al., 1994, Jost and Levet 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, 1997).

Medially, it adheres to the maxilla through the fibrous condensation of the nasolabial fold. 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, facing the superficial fascia. The latter has a much dense structure, its collagen fibers constituting a dense structure with homogeneous masses in which fibers (Macchi et al., 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, and shows that while reaching the parotid gland, the superficial fascia gives rise to the anterior capsule of the gland. This data is also acknowledged in the literature (Baek et al., 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 ressection. This aspect is however a novelty in maxillofacial surgery techniques and improves the results of the interventions at this level. The identification of the facial nerve in its quadrilateral intraparotid dissection, and especially the fascial tunneling of its terminal branches allows a better preservation of the nerve, yielding improved results for the patient (Ikoma et al., 2014). The decrease in the incidence of postoperative infections to less than 1% and the higher aesthetic results (Wong and Ahetty, 2017) prompt the use of the concept of the single surface layer demonstrated by us in parotid surgery.

In the parotid region, the superficial fascia presents numerous lobules. They are separated above SMAS by vertical fibrous tractus and subSMAS 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. Here the facial nerve, the facial artery enters to parotid gland and the external jugular vein exits the parotide.

In the genian region, SMAS's most important relationship is that of the maxilla and facial nerve branches. These nerve threads are accompanied by branches of the external carotid artery.

Their preservation is the greatest concern of a surgeon who intervenes at this level (Siemionow et al., 2006).

Nowadays, by preserving these neurovascular bundles, complete facial transplants are possible.

The classical theory of the origin of parotid fascia being derived from the deep facial fascia is invalidated by the continuity of its anterior layer with the platysma muscle fascia.

The superior part of the cheek makes the transition to the zigomatic region. The morphological, macro and mesoscopic differences between the two regions are clear and presents "as a border strip".

2.1.5.6. *Masseteric SMAS*

Parotid masseteric SMAS is well personalized and may contain some muscular fibers and nerves fibrous tunnels (Gola, 2005). It covers the parotid gland and masseter muscle (Pidgeon et al., 2017). Riolan, in “Le Double”, signaled “portio musculi cutanei supra parotidem ad aurem ascendentis” (Riolan, 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 inferiorly to the mandibular angle and in the 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. 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 dissect; 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 paracomisurally and pulls it back in the smile. This muscle develops in the SMAS thickness, before the masseteric aponeurosis, but without inserting it.

SMAS forms together with the skin a functional, tegumentary, adipose and neurovascular unit, physiologically inseparable, the cervicofacial cutaneous muscular aponeurotic unit (Zhang et al., 2013). On axial CT, SMAS appears as a relatively hypertensive, tortuous line between superficial fibrous tissue and deep hypodense adipose tissue. Quantitative measurements performed on CT by researchers (Macchi et al., 2007, Macchi et al., 2010) showed regional differences in thickness of the superficial layers of the face. We have also used MRI and CT images to determine morphological aspects of other muscle. Quantitative measurements were made on the representative microscopic sections taken with the microscope using an image acquisition system (a video camera connected to a PC). Further, we used the PRODIT 5.2 professional program. 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, 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 anatomoradiological 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 towards adjacent regions.

Also, masticatory movements define important force vectors in this area. In facial nerve damage, these are greatly damaged and require specific reconstruction techniques (Pippi, 2014).

2.1.5.7. ***Labial SMAS***

SMAS relationship with the nasolabial groove is still controversial. Literature studies (Mitz and Peyrone, 1976) described anterior continuation of SMAS into the upper lip, overlying the muscular layer. Barton (Barton, 1992) described a thin fascial layer covering the zygomatic muscles and extending into the upper lip, but they do not identify the subcutaneous extension of SMAS.

Pensler distinguished a SMAS layer which is medial to the nasolabial groove (Pensler et al., 1985), whereas Yousif consider that there is an adipose supraSMAS layer on the cheek and upper lip (Yousif et al., 1994).

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

- cutaneous, with a denser profound part, acting as 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 region: depressor labii inferioris, depressor anguli oris, platysma, mentalis.

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

Anatomy studies conducted with focus on superficial muscular and fascial structures support the Duchenne’s statement of 1862: “the law governing the expression of the human face can be discovered by studying the action ofthe muscles” (Duchenne, 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). 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 one hand, SMAS conveys the contraction of muscles along the parallel network toward the skin area, and on the other hand it transmits the resulting effect on a perpendicular direction towards the skin, through fibrous expansions from SMAS to the skin. Lifting of the upper lip for facial rejuvenation is based on these features of the regional muscles (Bai-lin, 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 robust adherence 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 of the almost non-existance of the supraSMAS adipose layer. Being composed of dense connective tissue the superficial and deep fasciae lose their elasticity.

2.1.5.8. Superficial planes of the anterolateral cervical region

The most common practical implication of the superficial layers of the neck is in facelift. Cervical aesthetics combines the appearance of a well-defined jaw line, an appropriate cervicomental angle, and visible shapes of sternocleidomastoid muscles and trachea (Liu et al., 2012). In contrast to the ideal features are: excess adipose tissue, skin, important relaxation of neck suspension structures and unfavorable skeletal features such as micrognathia and hyoid malposition.

Subcutaneous adipose tissue should be represented in a precise amount to create smooth outlines and transitions from one structure to another. Platysmal strips, wrinkles and skin folds should be removed.

The more a patient has an anatomy closer to the aesthetic ideal, the easier it is to predict the outcome of a reconstructive surgery.

Excess fat tissue leads to the appearance of a "hard" neck and involves particular difficulties in cervical lifting surgery.

The subcutaneous cervical adipose layer diffuses superiorly, in platysma leyer in all the cervical region. Between the anterior fascicles of the digastric muscle is another layer of adipose tissue covering the two miliohioid muscles.

Any of these layers of adipose tissue may become gravitationally displaced with the decrease in the tonus of the platysma muscle and the weakening of the mandibular ligament.
High cutaneous laxity associated with subcutaneous fat accumulation can cause "turkey" neck deformation (Fedok et al., 2014).

In the lower part of the face, the SMAS dissection allows the unitary lifting of the skin, subcutaneous tissue and platysma muscle. In the middle of the face, the deep subcutaneous *plane extends above the orbicularis oris and zygomatic muscles, independent of the zygomatic fat ped. Therefore, through the SMAS dissection, the skin, the subcutaneous tissue, the zygomatic fat ped and the platysma muscles can be raised unitarily.*

The most acute interpretation problems raised the controversial existence of the superficial cervicofacial musculofascial system, our study attempting to clarify its anatomical features.

Platysma and lower facial muscles are covered by a conjunctive layer that can be equated with the superficial fascia. It behaves identically to the mammary gland fascia or to the dartos muscular fascia of the scrotum or major labia and could be named the superficial musculo-fascial system.

SMAS is an anatomic entity made up of a fibromuscular layer covering the mimic muscles and parotideomasseric fascia, but also a surgical entity because it is an important landmark in facial reconstructions. In addition, the platysma - SMAS – superficial temporal fascia - frontal – galea aponeurosis - occipital represents a functional unit that defines individual facies.

Superficial fibrous tissue is crossed by vertical fibrous formations that connect the deep dermis to the superficial part of SMAS.

The profound fibrous layer has oblique oriented fibrous septae, which connect the deep face of SMAS to the deep fascia. The interconnection of SMAS through fibrous septa from the above and underlying layers creates a 3D network that modulates the transmission of muscle contraction to the skin.

Anatomical and surgical dissection in adults shows that platysma muscle continues with facial SMAS. SMAS was considered a fibrous degeneration of the platysma muscle (Stuzin et al., 1992) or an extension of the cervical superficial fascia (Gosain et al., 1993).

The platysma muscle is the subject of significant morphological variations in terms of its thickness, interleaving in connective tissue and its enlargement. It may reach up to the zygomatic arch or even to the orbicularis oculi muscle (Sharpey et al., 1923).

The modern techniques of cervicofacial or cervical rejuvenation are based on anchorage techniques of the platysma muscle at the tymapanomastoid fascia. This structure is the facial extension of the platysma fascia, also known as Lore's fascia. Cervical lifting techniques based on the existence of cervical SMAS structure are combined with dissection above or under the platysma muscle. The technique that involve the partial resection of the anterior fascicle of digastric muscle combined with the restoration of the platysmal strips and platysmoplasty allows the reconstruction of ptotic neck aesthetics in a single procedure (Athanasiou et al., 2014, O'Brien et al., 2018).

Our results coincide with those in the literature by the fact that the platysma muscle appears from the cervical lamina and the mandibular extension. Its evolution is very variable, occupying the lower part of the parotid and cheek regions.

The continuity of the platysma fascia with the superficial fascia of the face explains the superior results of the modern cervicofacial rejuvenation techniques. The extension of adipose tissue from around the platysma to the face explains the migratory possibility of inflammatory, infectious or tumoral processes between the two regions. We intend to continue the cervical SMAS study by performing more dissections in younger corpses and modern imaging techniques, such as functional MRI.

The superficial fascia of the neck forms the anterior sheet of the platysma fascia and and the anterior sheet of the submandibular gland capsule under the menton.

In the lower part of the face, SMAS dissection allows the unitary lifting of the skin, subcutaneous tissue and the platysma muscle. In the middle part of the face, the deep subcutaneous plan extends above the orbicularis oris and zygomatics muscles independent of the zygomatic fat pad. Therefore, through the SMAS dissection, the skin, the subcutaneous tissue, the zygomatic fat pad and the platysma muscles can be raised unitarily.

2.1.5.9. Spatial relations of the terminal branches of the facial nerve with the facial structures

The extracranial segment of the facial nerve begins at the stilomastoid orifice, where it leaves the skull. It reaches the facial quadrangle where, for identification, we used the bone markers presented earlier. It penetrates the parotid gland at the level of a point located at the union of superior 1/3 with inferior 2/3 of the line drawn through the posterior edge of the mandibular rami that continues anteriorly through the parotid space. Initially, it is located out of the gland, then into the gland where it forms pes anserinus facial nerves, from which two primary terminal branches are detached: temporofacial, more voluminous, with horizontal direction and cervicofacial, thinner, almost vertical.

The examination of the anatomical specimens has demonstrated the great variability of the division of the primary trunks of the facial nerve. Ramification of the facial nerve can not fit into a rigid pattern, ~~with~~ as it presents varaible distribution patterns. The anterior dissection of the extracranial facial nerve complex allowed us to consider that only the initial bifurcation corresponds to anatomical data, the secondary, but mostly the tertiary ramifications, have numerous variations in number and course.

Regarding the spatial distribution of the branches, the microdissection allowed the identification of two groups of regions with significant stratigraphic differences.

Due to the special topographic situation of the terminal branches of the facial nerve, the superficial facial muscles receive innervation throughtheir deep face and the deep facial muscles through their superficial face.

In the posterolateral regions of the face we identified, from the surface in depth, the following layers**:**

- cutaneous,
- subcutaneous adipose tissue,
- superficial fascia,
- the branches of the facial nerve,
- deep, parotid, masteterin or temporal fascia,
- the musculoglandular layer, represented by the masseter muscle and/or the parotid gland.

In the anterior regions of the face we identified, from the surface in depth, the following layers:

- skin, epidermis and dermis, with the thicker profound part, serving as insertion for the muscles of facial expressions,
- the subcutaneous adipose layer,

- superficial facial muscles plane, different by studied region:

  - *infraorbital region:* zygomatics muscles, levator labii superioris,

  - *oral region*: zygomatics muscles, rizorius, orbicularis oris, levator labii superioris and levator anguli oris muscles and modiolus,

- *menton region*: depresor labii inferioris, depresor anguli oris, platysma,
  - the branches of facial nerve plane,
  - the deep plane of the facial muscles, different from one region to another:
- *infraorbital region*: levator anguli oris,
- *oral region*: levator anguli oris and buccinator,
- *menton region*: mentalis.

The major interpretation problems raised the controversial existence of the superficial cervicofacial musculofascial system, thus our study attempted to clarify its anatomical features.

Platysma and lower facial muscles are covered by a conjunctive layer that can be equated with the superficial fascia. It behaves identically to the mammary gland fascia or to the dartos muscle fascia of the scrotum or major labia and could be called the superficial musculo-fascial system.

Our studies reveals that the aponeurotic portion of the superficial musculo-fascial system is individualized under the subcutaneous adipose layer only in posterolateral areas of the face where there is a deep, well-tensioned fascial support, as is the case of the parotidomasseric, buccal and frontal lateral regions.

As a result, the spatial disposal of frontotemporal and zygomatic branches recommends their protection through interfascial dissection in facial surgery (Salas et al., 1998).

The mesoscopic dissections performed led to similar conclusions to those in the literature with respect to tensioning the fascial part of the superficial musculofascial system. It is tensioned in every directions:

- superiorly, by frontal and orbicularis oculi muscles,
- anteriorly, by major zygomaticus muscle and modiolus,
- inferiorly, by platysma muscle and unites with superficial cervical fascia,
- posteriorly, by galea aponeurotica and superficial leyer of temporal fascia, after which it attaches to tragus and mastoid (Jost and Levet 1984, Pensler et al., 1985, Morales 1991, Barton, 1992).

Anatomical studies conducted with focus on superficial and muscular structures reminded us of Duchenne's statement in 1862 that "*the law governing the expression of the human face can be descovered by studying the action of the muscles*."

In this context, SMAS, more precisely the muscle-fascial sheath which continues the frontal muscle to platysma plays an essential role, that of a "facial muscular 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 transmits the contraction of the muscles along the parallel network to the skin plane, and on the other hand it transmits the resulting effect in a direction perpendicular to the skin through fibrous expansions from SMAS to the derm.

That is why the human face has a great ability to express different nuances of expression. The profoundly important coating, the "deep neck sphincter" (sphincter colli profundus), includes all the subcutaneous muscles grouped into 3 orbicular muscles. They have bone attachments necessary for the active remodeling of the orifices in expressing emotions (Hueston 1990).

Outside of this powerful muscular cover, there is what Jost and Levet have called ”primitive platysma”. It covers the face and neck, and in monkey is strongly attached to the parotid fascia and ear cartilage. In humans it is represented by 4 muscles: platysma, rhizorius, mentalis and posterior auricular united by parotid fascia.

This distinctive and functional sheath does not have bone attachments, being the layer that is surgically tensioned in lifting.

2.1.5.10. Congenital facial atrezia

The facial dermis is fixed to the bones through a fibrous support, which contains ligaments and superficial fascia, and at the same time allowing the movements and facial gestures. This attachment must also resist external forces such as gravity and traction.

The ligaments, which fix the superficial fascia to the skeleton, can divide the face into several distinct regions. Three of them are in the mental and infrazygomatic regions: chin, lateral side of the chin (Jost and Levet 1984).

Besides these regions, we can include those of lower eyelids, upper eyelids, infratemporal and frontal.

In these regions, the ligaments between the posterior aspect of superficial fascia and facial skeleton “put in quarantine movements caused by muscle contraction, at least in young adults, so that movements of superficial fascia will not be transmitted to neighboring regions”.

Outside the classical division of the face in three zones – superior, middle and inferior – one can be added based on aesthetic, anatomical and functional considerations. Anatomical pattern of ligamentous attachment of the superficial fascia to the facial skeleton defines the limits that divide face in several regions. Three of these are parts of what is seen on the outside such as: the cheek, lateral cheek, pre- and infrazygomatic sides medial cheek, in addition to the other regions: lower eyelid, forehead and upper eyelid. Congenital atresia of the facial nerve has a different impact on these parts of the face, both in terms of morphology and in of appearance of clinical signs over time. In severe cases of bilateral congenital atresia, its clinical manifestation is more severe and presents earlier.

The weakening of connecting tissue through muscular shortening and stretching determines the displacement on different directions depending on the configuration of each muscle, separately.

There are multiple movement vectors in facial aging because each muscle of facial expression has its own connection direction of the lax tissue. The correction of each vector displacement requires a proper fixing vector. The presence of Moebius syndrome associated pathologies speeds up appearance of connection tissue degeneration, leading to earlier clinical manifestations. Fortunately, SMAS concept usage provides increased opportunities for controlling the direction of lifting. We can hence say that the face is made up of three distinct functional morphological regions: median, medial, lateral-posterior (Berkovitz and Holland

2002).

The medial region of the face includes superficial and deep layers around the face openings, having the shape of a butterfly with raised wings. The main feature of this region is the fixityof superficial structures to the deep onemore precisely powerful, flared muscular insertions on the deep part of the dermis.

It can be said that it is the visceral segment of the face. Most muscles play the role of sphincter of the openings they surround, while the others represent their functional extensions. The medial part of the face is predominant in individualizing the facies and hence, the relational life of each individual.

Therefore, we call it the "relational" part of the face, and we can divide it into four subregions: orbital, nasal and oral. Although muscles are different in each of the regions, there are some common considerations, in terms of stratigraphy, quantity and quality:

- *the skin gradually thins as we move towards the mediosagittal area;*
- *the same behaviorhighlights in the subcutaneous adipose tissue,the difference being that the layer beneath the superficial fascia is almost nonexistent;*
- *superficial and deep fascia lose their elasticity, being made up of dense connective tissue.*

In some situations, both fascia are reflected with one another and can adhere to the periosteum region. This happens periorbitally (ligamental adhesion where it is impossible to perform a deep dissection) at the lower edge of nasal cartilage and at the midline union of the two nasal wings (Bosse and Papillon 1987).

The medial region (intermediate) of the face is a paired region, mainly characterized by a high degree of mobility of the superficial tissue compared to deep layers. It is bounded superiorly by the zygomatic arch (Delmar, 1994) and inferiorly by mandible arch. Laterally, it spreads to the parotid gland capsule and medially to a linewhich joins the angle of the mouth with the lateral edge of the nasogenian groove.

The exacerbated mobility of these regions, compared to the other two, is due to the fact that it contains two fat peds (malar and jugal)of considerable size.Connective fibers (Gardetto et al., 2003) of the superficial fascia of these fat pads adhere strongly to the deep layer of the dermis. In depth, they are "anchored" only by the cutaneous insertions of zygomatic muscles.

This region is also one of "passage", SMAS (Gola, 2005) realizing connective tunnels through which branches of the facial nerve, Stenon's duct and branches from facial vessels cross from the lateral part to the medial part or vice versa. Duplication of SMAS with the formation of tunnels for nerves and vessels is done through a trans-versely oriented connective tissue (Gosain et al., 1993), while the superficial layer, whose origin is orientated from superior to inferior, isin the direction of the traction vectors of zygomatic and infraorbital insertions.

Another important role of these regions is to form an interconnection between the medial and the lateral region of the face, providing an additional support to actions that occur at this level. Thus, it actively intervenes in far-reaching facial expressions, such as making a wide smile. This stands out when the region is developing an inflammatory or tumoral process, which disrupts its functionality. In this case, it can cause active facial dysmorphism (in an attempt to express facial gestures) or passive dysmorphism through retraction of tissue and loss of anti gravity support (Hughes and Salinas 1999).

The role of the most powerful masticatory muscles (temporal, masseter, buccinator) is amplified, through a leverage mechanism, by the connections of their insertion regions with the perioral one (Piccioloni et al., 2016).

Mimics in this area can occur only on its boundary where the muscles of facial expression take insertion.

The p*osterior lateral region of the face is a paired region, fixing the face to the exoskeleton. Here lie strong ligaments and ligamentary adhesions that, on one hand anchor soft tissues of the face and, on other hand, delimit it by the neighboring regions: frontal, temporal, lateral-cervical and cervical.*

Being particularly visible from profile, this region intervenes indirectly in creating facial expressions. At this level, in deep plan masticatory muscle inserts on mandibular ramus. The region extends superiorly to the zygomatic arch, practically covering the infratemporal fossa. Laterally and posteriorly, one can make up the parotid region, while the deep fascial layer is made up by parotidian masseteric fascia.

2.1.6. Conclusions

At the junction level of the craniofacial and cervicofacial areas, there are muscular transfascial attachments, fibrous adhesions and ligaments, which fix SMAS to the profound layers. Due to the stabilizing role, we have described all these compact structures as the sustentaculum facies. The periorbital adhesions represent a support point which converges toward all the adjacent ligaments orientated in the three-axial space, and together with the zygomatic ligament form the upper portion of sustentaculum facies. They are the main anti-gravity suspension mechanism of the face. The infraSMAS plan is connected to the profound layer by adhesions on one hand, and by a connective conjunctive adipose tissue on the other. The latter is crossed by the neurovascular branches, a port-vessel tissue and a portnerve similar to one from other regions. The percentage decrease of collagen fibers or of the sliding fibroadipose tissue during aging explains the decreasing of the functional capacity followed by the face fall.

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 subcutaneously as a fat layer, just under the posterolateral areas of the face (parotideo-masseteric, oral and frontal), where there is a deep fascial support, functionnaly tensed.

Musculofascial superficial aponeurotic segment in masseteric region is distinguished in the subcutaneous fat layer only in the posterolateral areas of the face, where there is a deep fascial support, functionnaly tensed. Collagen fibers are dispositioned orderly on successive longitudinal and transverse planes, or have individual structure, forming layers of varied shapes and sizes, most obviously at the level of the modiolus. Muscle fibers belonging to the superficial layer of the skin that form SMAS in some areas, leave or cross to the deep surface or the osteoperostic plane.

Masseteric SMAS is a dense lamina, particularly with collagen fibers, and rare muscle fibers. SMAS layer has a mixed structure, with very small quantitative differences between connective tissue and muscles. The masseteric fascia is a surgical entity as it is has an important function in facial reconstructions.

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. The 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. In upper lip, SMAS has a mixed structure with quite small quantitative differences between the connective and muscular tissue. SMAS also realizes 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 for various types of facial surgery.

The clinical and morphological changes that occur in Moebius syndrome are strongly correlated with a particular structure located on the face and called SMAS. In Moebius syndrome, the mimic muscles of the central regions of the face have a significantly decreased role. Through fibrous extentions in the medial region, SMAS determines the robust adherence of the fat pad at this level. This provides the contour of the cheek, and SMAS has an active role in raising the soft tissues of the cheek. The anterior capsule of the parotid gland is formed by SMAS. From this level it is continued posteriorly and laterally with the fascia of sternocleido-mastoid muscle and anteriorly and medially with fibrous tunnels for facial nerve branches. At the supraorbital level, there are strong adhesions between the ligaments of superciliary arches and the dermal insertions of the superior fascicle of orbicularis oculi muscle, which are becoming increasingly lax. Continuities of this fascia with the surrounding fascia – frontal, cervical, temporal – are due to pathological changes associated with Moebius syndrome.

2.2. ANATOMICAL SUBSTRATE OF PHONATORY APPARATUS

2.2.1. State of the art

Professional singing requires some individual qualities but also a long time of specialized training.

Based on our personal experience so far, we can say that the oropharyngeal pavilion is morphologically and functionally adapted to certain vocal needs / requirements.

Concurrently with these reflex phenomena known in the literature (Husson, 1960) emerges a complex adaptation reflex of the pavilion to the glottic reflex changes. We noticed and discussed this reflex in our studies and called it pharyngo-recurrent reflex. These observations are the basis of the motivation and necessity of the proposed study.

This statement is based on the fact that experienced singers make these adaptations much faster, in a reflex manner, when compared to ~~than~~ debutants that require a warm-up and vocal concentration.

Studies on the oropharyngeal pavilion have the following specific objectives, defined in order to reach the general objective :

- detecting so-called hot points within the pavilion in terms of the intensity of functional stimulation.
- making a starting point for researching of other types of artistic voice, both different from the point of view of the individual ambitus and the type of voice training performed

2.2.2. Scientific context and motivation

Accurately defining the voice forming mechanisms ~~it~~ has always been the interest of researchers in the fields of anatomy, physiology, otorhinolaryngology, phoniatry and canto.

This study aims to find out what are the anatomical and functional variations that allow some individuals to have special vocal abilities and to improve them. Through vocal abilities we refer to the pre-existing conditions necessary for an individual to perform in different forms of singing, for both genders.

It is very important to note from the beginning that this study addresses to subjects who do not have a pre-existing pathological condition that can influence the quality of the voice.

This research attempts to highlight the morpho-functional characteristics of this antenna type apparatus in order to establish intrinsic factors that actively and passively participate in the canto voice.

The studies we have done so far aim to identify the role of the oropharyngeal pavilion in the formation of the artistic voice both in opera and theater. It is known that the training of an actor involves hours of methodology and practice of both diction and singing.

The phenomena that contribute to the adaptability of this space influence reflexively the activity of the vocal muscles and, implicitly, the adaptability of the vocal cords.

The basis of this latter mechanism is a series of rhythmic motor patterns acquired after a period of about 7-10 years of vocal training.

The main objective of this study is to highlight the qualitative and quantitative changes of the sound passage column made by adaptive phenomena occurring at the oropharyngeal pavilion, the laryngeal glottic floor and the infraglottic filter.

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.

2.2.3. Materials and methods

2.2.3.1. *Anatomical study*

The study was conducted on a number of 7 anatomical parts preserved by phormaldehyde, at the Institute of Anatomy "Ion Iancu" of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi. A laryngoscopic exam was initially performed on a group of 7 adults, with assessment of the macroscopic appearance of the endolarynx and the dynamics of the vocal cords, in order to exclude those with associated pathologies of the phonatory apparatus.

The subjects indicated that they do not have preexisting laryngeal pathologies and do not accuse dysphonia or other voice changes. They also denied smoking, a habit that affects the larynx and influences the results.

In the second step, precision measurements were obtained through MRI images, which were used in different formulas.

We then dissected and highlighted the cartilagenous, muscular, and ligamentous structures of the larynx, and then took measurements at this level (Fig. 2.2.1.). The obtained values were used in statistical calculation formulas.

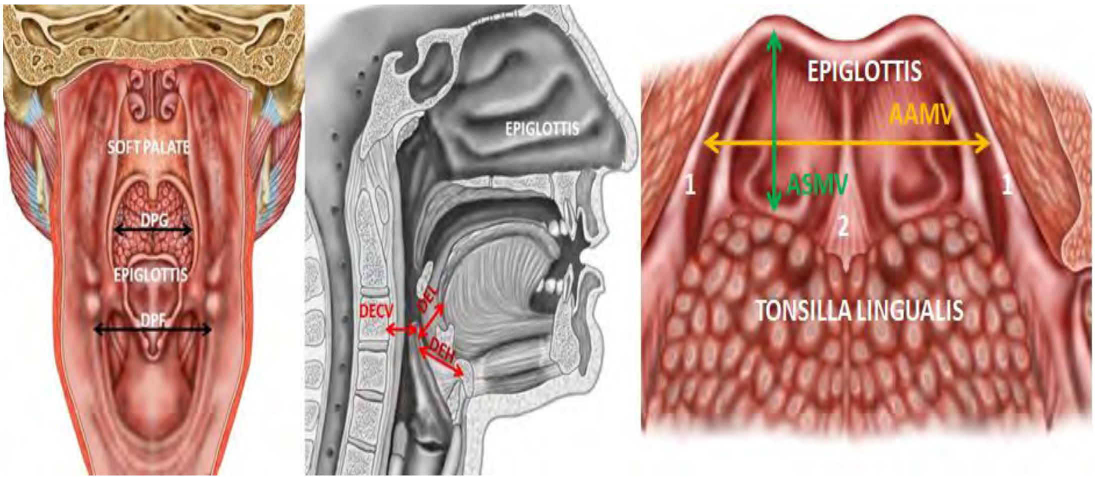

Figure 2.2.1. Measurements of buccopharyngeal pavilion.1=plica glossoepiglottica lateralis, 2=plica glossoepiglottica medialis

In the macroscopic, clinical and radiological anatomy study, a statistic was performed using the metric values of the laryngeal ligamentary apparatus involved in the phonation and the volumetric measurements of the infraglotic filter (volume of the infraglottic floor).

2.2.3.2. *Radiologic study*

The present research is an anatomo-imagistic study, which aims to highlight the morphofunctional characteristics of this “antenna apparatus” (velo-oro-pharyngeal-glottic vocal formant), in order to establish the intrinsic factors that actively and passively participate in the realization of the soprano voice.

More precisely, our research team performed CBCT measurements on the mobile anatomical structures involved in the delimitation of the superior voice formant: the palatine veil, palatine veil pillars, epiglottis, valleculae, base of the tongue and the oropharynx. We also determined the volumetry of the entire functional space, known in the specialised-literature as buccopharyngeal pavilion.

We noted the position of these structures in relation to the skeletal projection and took metric measurements of the distances between the muscular and ligamentar structures. Volumetry is addressed to both the entire space and its subdivisions (ventricular spaces).

The data processing software enabled us to have a 3D reconstruction of the space and to compare the features of the new pattern with the results of the linear measurements made on the 2D sections and its modeling in a 3D printing. This is useful in analyzing the shape of the entire superior formant of the harmonic voice.

We conducted this study on a group of patients who had agreed and were willing to undergo this investigation, having no associated pathology that could affected the vocal quality. This was certified on the basis of the medical history and clinical examination.

Each person was subjected to CBCT investigation both in the moment they were pronouncing basic vowels in phonetics, such as "Ə, I:" (which we call: phonatory state I and phonatory state II) and when they are in phonatic rest (just mimicking the phonation). When pronouncing vowel "I:" the larynx is in the highest position, while when pronouncing the vowel "Ə" the larynx goes to the lowest position.

The whole investigation lasts 15 minutes, out of which, the exposure is 42 seconds.

The group contribuing to the preliminary report consisted of 3 subjects with soprano voice phonatory characteristics, as assessed by canto teachers. The first of the subjects has medium vocal practice level (7 years) while the third one ~~is~~ was an experienced soprano soloist having over 20 years of practice. The second subject has also vocal features of soprano, but with a vocal practice of over 15 years in theater voice. Theater voice requires vocal exercises similar to opera voice but also vocal techniques, such as for professional actors.

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 will undergoe shape changes in response to the intensity of the functional demand. To this regard, an anatomical variation present in subjects with special vocal abilities will be more evident in people with long vocal practice.

The common morphometric and volumetric characteristics of our subjects were considered anatomical variations that empowered each of them with vocal abilities.

The CBCT investigation allows us to reproduce a negative model of the third vocal formant and to make accurate measurements.

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

Cone-beam Computed Tomography (CBCT) is a state-of-the-art imaging technology that allows three-dimensional viewing of scanned areas. Using a conical x-ray beam, the CBCT scanner needs a single rotation around the patient to take hundreds of images of the area of interest, which are then reconstructed using an imaging software to obtain the patient's

3D virtual model.

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. 3D conical beam technology has reached new levels and has become the reference point in maxillofacial imaging but has never been used to explore vocal training and modulation mechanisms.

As the name suggests, CBCT generates X-ray conical beams and the images are obtained in a single rotation through an image intensifier of a flat panel detector resulting in reasonably low radiation dose levels (Chan et al., 2010, Scarfe and Farman 2008, Arai et al., 1999).

The resolution and therefore the details of the CBCT view are determined by the individual volume elements or voxel produced by the volumetric data setting. In the CBCT view, the voxel dimensions depend mainly on the size of the pixels in the detector area, unlike those in the CT, which depend on the thickness of the "slice". The resolution in the detector area is submillimetric.

Thus, spatial resolution of CBCT is higher than that of CT (Scarfe, 2008). In the specialised-literature, CT and CBCT were compared to determize sizes at operating situs levels and CBCT measurements were found to be the most acurate (Al-Ekrish and Ekram 2011, Kobayashi et al., 2004, Loubele et al. 2008, Naitoh et al., 2009, Mah et al., 2010, Nomura et al., 2010).

The great advantage of CBCT equipment (Hein et al., 2002, Ludlow and Ivanovic 2008, Loubele et al., 2009, Roberts et al., 2009, Ruivo et al., 2009, De Cock et al., 2012) is represented by an increased flexibility with a wide range of clinical applications. Typically, these devices have the advantage of allowing the usage of different sizes in the exploration protocol, different heights and diameters, , depending on the clinical utility. The amount of data acquired is smaller and thus easier to archive (Liedke et al., 2009, Lofthag-Hansen et al., 2011, Razi et al.,2014).

The major advantage of CBCT imaging in the oral and maxillo-facial region is accessibility, easy handling and real information resulting from multi-plane and three-dimensional reconstructions with a single, low dose irradiation exam.

- ***CBCT examination protocol***

The CBCT equipment used was PlanmecaPromax 3D Mid (Planmeca OY, Helsinki, Finland). Scanning was performed by selecting a 20 x 17 mm FOV and the following exposure parameters: 65 kV, 8 mA, 13.9 seconds and 0.4 x 0.4 x 0.4 mm voxel size. The parameters used are chosen so that the accuracy of the volumetric evaluation of the airway and the oral cavity is correlated with a minimum irradiation dose. Scanning is done by exposing the patient to the equipment in the seat, and both x-ray source and sensor make a complete cycle of rotation around the cranial-cervical region, resulting in a volume acquisition on which the necessary reconstructions can later be done. Each patient will sign an informed consent explaining the purpose of the study.

My research team has been involved in establishing the appropriate protocol. After repeated previews we chose the device's pre-established protocol for exploration on a 12.5

cm section. We decided on this particular section because this exploration allows us to obtain images of the buccopharyngeal pavilion from glottis to gard palate as well as antero-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 skeletal projection or the starting point for linear measurements.

Image exploration was performed while maintaining the phonic position of the oro-velo-pharyngeal-laryngeal formant (emitting a sustained sound during the acquisition of images).

Initial and final reconstructions are performed by the Romexis 4.4.2 software (Planmeca, Helsinki, Finland). In order to achieve axial, coronal and sagittal sections, we performed CBCT reconstructions of 1 mm thickness at a distance of 1 mm

2.2.3.3. Volumetric analysis of pharyngeal airways

The captured volumes were opened in the Romexis 4.4.2® software (Planmeca, Helsinki, Finland). in a standard viewing window for reconstructions in the three planes: axial, coronal and sagittal. All these evaluations were performed in a room with poor light by 3 specialists experienced in imaging.

The volume of each oral cavity and pharynx is measured using the same software.

To calculate the volume, we first defined the region of interest by using the segmentation tool. At the level of the axial sections we have selected the pharyngeal boundary segmentation tool starting from an imaginary plane that passes through the base of the vallecula and reaches up to an imaginary plane located behind the nasal choans. Axial sections at a distance of 0.5 mm will be used to increase the fidelity of the calculated volume. Before creating the volume at the level of each pavilion, we decided on the necessary standard presets.

A 3D volume module can be used to calculate the perimeter and the volume of a non-uniform anatomical region with PlanmecaRomexis®. The axial sections at the interest region level can also be colored so that the color of each region depends on its size.

The data obtained was processed and interpreted to define parameters that could characterize the studied structures in order to highlight morphofunctional variations that can be correlated with eighter a certain individual vocal ability or a common ability to the three subjects, or both.

2.2.4. Results

2.2.4.1. *Anatomic study*

Measurements were made on: DAP - glotic anteroposterior diameter, DMG - glotal maximum diameter, LCV - vocal cords length before and after mucosal removal, DC - maximum distance between vocal cords, LIT - interaritenoidian distance, HIG - the height of the infraglotic floor, VIG - the volume of the infraglotic floor and the MIG - the length between the vocalprocesses (Figs. 2.2.2., 2.2.3., 2.2.4, 2.2.5, 2.2.6., 2.2.7.).

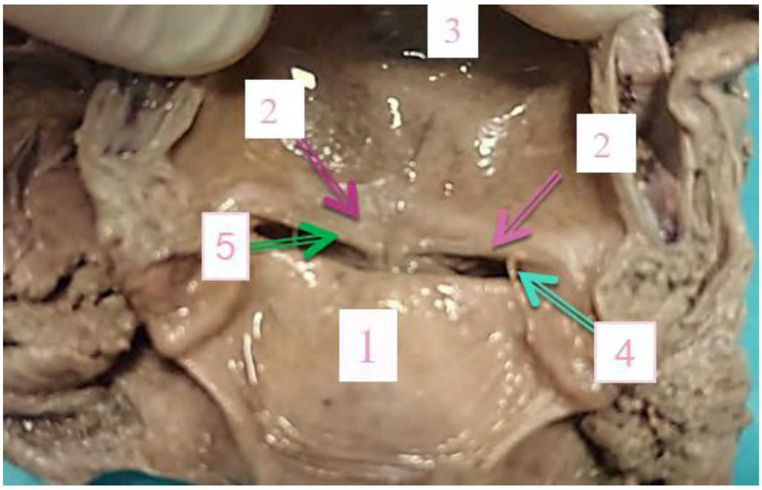

Figure 2.2.2. Dissection specimen: 1 –supraglotic floor, 2 –true vocal cords, 3 –infraglotic floor, 4 – false vocal cords, 5 –laryngeal vestibule

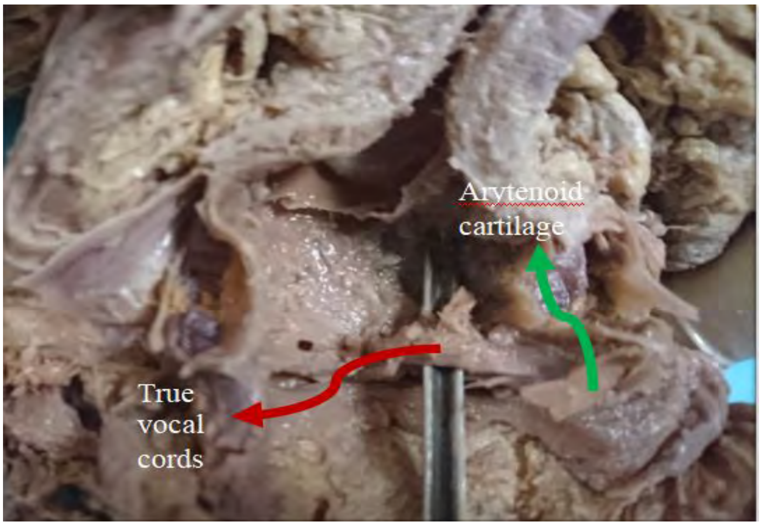

Figure 2.2.3. Dissection and measurement of the vocal folds

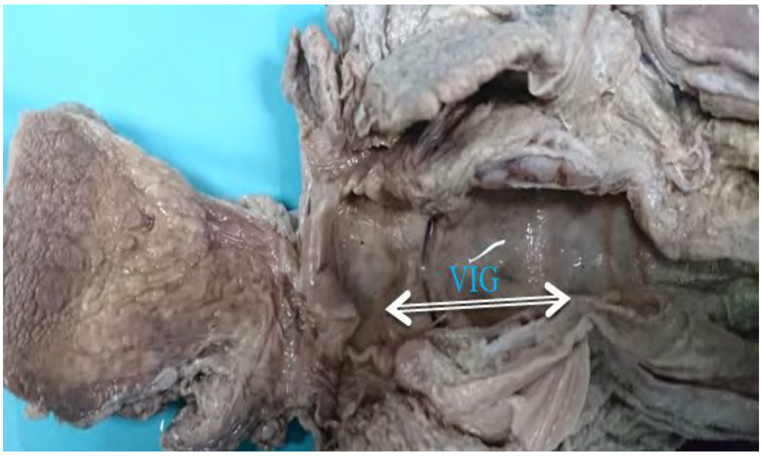

Figure 2.2.4. Descriptive image of the measurement of infraglotic volume

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

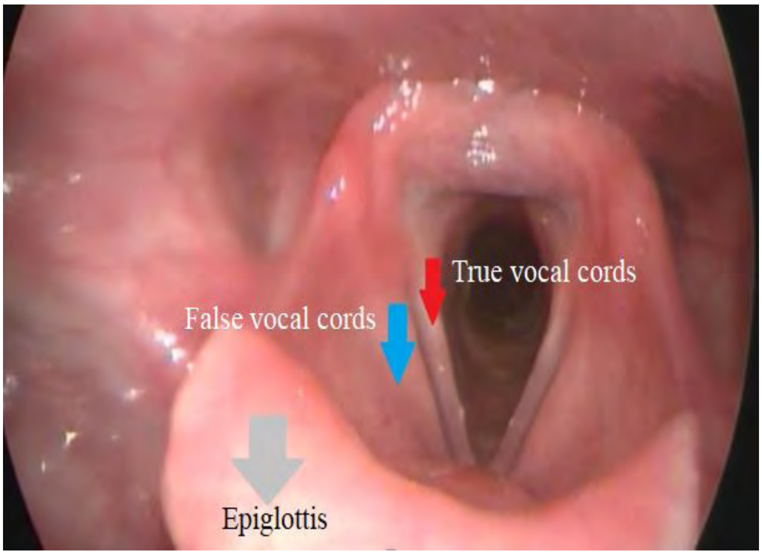

Figure 2.2.5. Laryngoscopic examination which shows no pathological changes

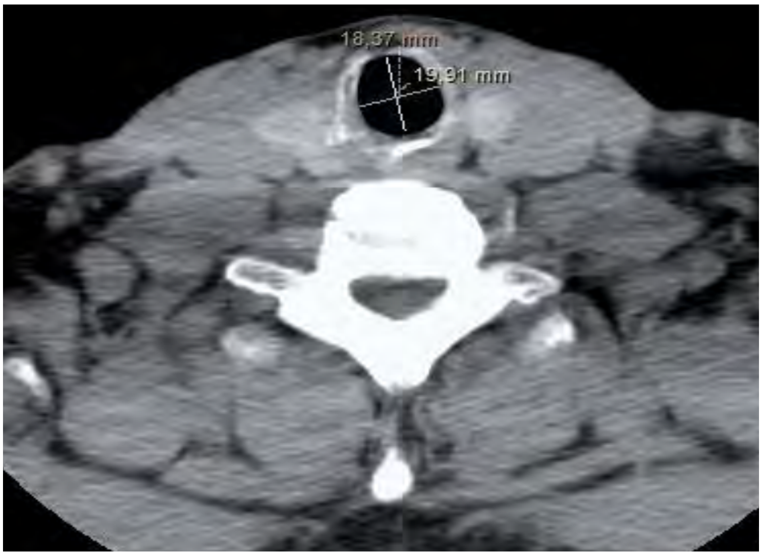

Figure 2.2.6. Forced exhale - closed vocal folds, observing through their transparent arytenoid cartilage

For measurements on dissection parts, the DC / LCV ratio has an average value of 2.53 with a variation range of 24.05%. DAP / LCV has an average value of 2.4 with a variance of 11.27%. DAP / LIT has an average of 2.4 and a variance range of 6.4%.

Between male and female gender the highest morphometric differences were found in the VIG (20%), HIG (50%) and the DMG / HIG (3.2%).

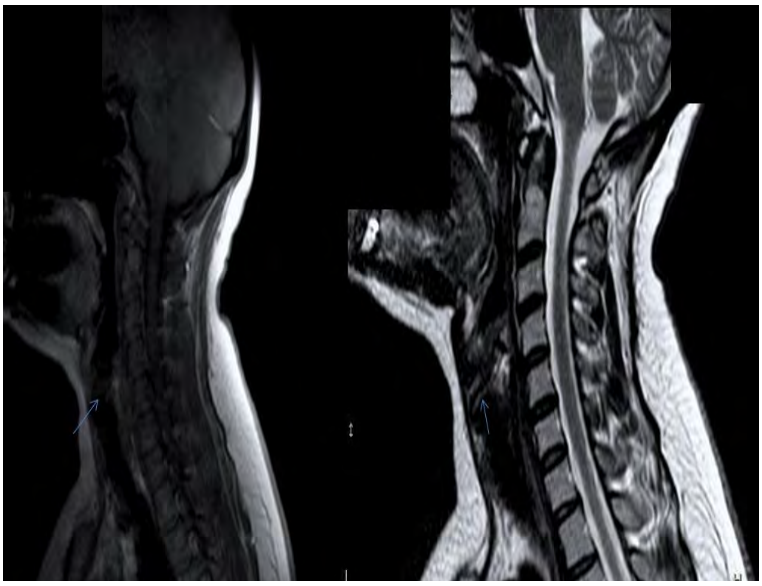

Figure 2.2.7. MRI Highlighting the vocal folds

The same measurements performed by the imaging technique are presented in **Tables 2.2.1** and **2.2.2.**

Table 2.2.1 Measurements of the vocal spaces

Measured parameters	Female patients		Male patients	
LCV (lenght of the vocal folds)	Right 1.49+/-0.11 cm	Left 1.46+/-0.07 cm	Right	Left
GCV (thickness of the vocal folds)	Right 0.866+/-0.29 cm	Left 0.867+/-0.23 cm	Right 0.897+/-0.46 cm	Left 0.804+/-0.11 cm
HVC (height of the vocal folds)	Right 0.5+/-0.32 cm	Left 0.502+/-0.28 cm	Right 0.735+/-0.32 cm	Left 0.608+/-0.45 cm
DAT (arytenoid diameter)	Right 0.346+/-0.14 cm	Left 0.307+/-0.23 cm	Right 0.62+/-0.40 cm	Left 0.69+/-0.65 cm
DAP/DMG (anterior posterior glotic diameter)	1.863+/-1.5 cm		2.55+/-4.33 cm	
LIT (interarytenoid diameter)	1.64+/-0.67 cm		1.33+/-0.98 cm	
MIG (lenght of the distance between arytenoid vocal processes)	1.114+/-0.84 cm		0.99+/-1.05 cm	
Cricoid ring diameter	1.47+/-1.19 cm		1.99+/-1.23 cm	
HIC (infraglotic floor height)	0.996+/-0.12 cm		1.24+/-0.85 cm	
Infraglotic floor volume	0.988+/-0.54 mL		1.167+/-0.80 mL	

The ratios obtained between these values are as follows:

- LCV/VIG=10,48-19,1%
- DAP/LCV=4,16-9,38%
- DAP/LIT=8,34-12,16%
- LCV/VIG=1,9-27,27%
- DAP/LCV=9,09-11,03%
- DAP/LIT=10,49-13,37%
- LCV/LIT=12,3-16,29%
- DMG/MIG=15,91-30,92%
- DMG/VIG=12,72-25,04%
- LCV/LIT=16,8-29,6%
- DMG/MIG=12,12-67,09%
- DMG/VIG=6,25-31,25%.

Table 2.2.2. Ratios between vocal spaces measurements

RATIOS	FEMALE SUBJECTS	MALE SUBJECTS
LCV/LIT	0.90	0.125
LCV/VIG	0.014	0.0014
DAP/LCV	1.25	0.154
DAP/LIT	1.13	0.149
DMG/MIG	1.67	2.31
DMG/VIG	0.018	0.016

Relatively constant ratios between vocal cords and interarytenoid distance, and constant rations between vocal folds and the maximum opening of vocal processes demonstrate that proportions increase proportionally at glotic level.

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

These findings indicate a complete functional specialization of the glotic structures in phonation.

We have obtained ratios whose results are slightly higher than average, such as LCV / VIG with a variation of up to 131.57%, where we have a LCV of approx. 3cm, given that the female LCV is about 1.1-1.5cm (in this case, an inverse variation is observed, as the LCV increases, the VIG decreases, so the amplitude of a sound is not given by the LCV, but by its tension).

The DMG / 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. The bigger it is, the lower the voice quality - especially for singers. Narrow DMG and a small LCV determine the production of a sound with high timbre, with an increased f0, characteristic of female gender.

Broad DMG and an increase in LCV characterize male gender with the production of baritone voice and the fundamental frequency decreases.

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

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

An increased infraglotic volume can be compensated by an adequate respiration.

As a result of these measurements, we observed a greater variation in a set of ratios. Thus, there is a relationship of inverse proportionality between the parameters of each increased ratio. The values with a variation of less than 5% suggest a direct proportionality relationship (Fig.2.2.7.).

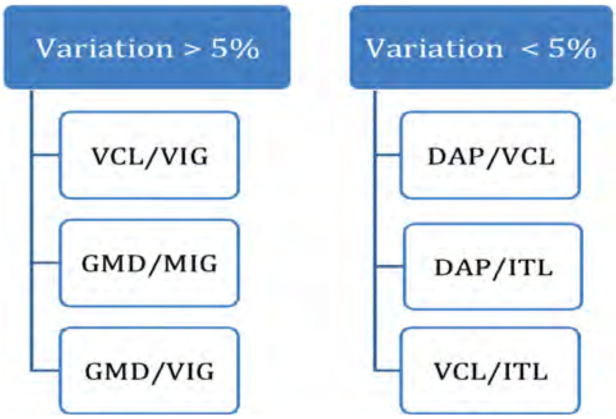

Figure 2.2.7. Lowest and highest percentual variations of the ratios

The results obtained were compared with the results of similar measurements made on molded parts. The ratios where major numerical differences were reported are:

- LCV/VIG (18,42-131,57%) - this difference is mainly based on the existence of 3 cm of vocal cords at the level of a molded piece
- DMG/VIG (28,57-121,42%) - the percentage varies depending on the VIG's high value (9.55), which may indirectlyindicate either an anomaly of the phonatory apparatus or the fact that it belonged to a person in the musical field (increased infraglotic volume allows to maintain a 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 to identify wether a parameter is constant or not from physiological point of view, with conclusions being drawn from current anatomical functional knowledge. Also, the morphometric study of the normal phonatory apparatus is important for differentiation from the associated pathology.

2.2.4.2. *Radiologic study*

The acquired data were interpreted starting from the measured values of the CBCT exploration of the subjects in phonatic repose. The following measurements were considered (Fig. 2.2.7.):

- the maximum opening of the oropharyngeal isthmus in the palatoglossal (DPG) and palatopharyngeal 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)
- skeletotopic projection of glottis (GP)
- 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)
- the volume of 3D reconstructed buccopharyngeal space (VFBS)
- changes in aspect of the buccopharyngeal space.

We identified the three subjects with S1, S2 and S3: S1 was the subject with medium canto practice, S2 was the subject with a voice practice in theatre, and S3 was an experienced soprano soloist with over 20 years of experience and practice.

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), in Tables 2.2.3.–2.2.12.

Table 2.2.3 DPG values in mm

Subject	**MLH**	**mLH**	**MP**
S1	**34.80**	**48.01**	**42.00**
S2	**39.25**	**37.22**	**45.61**
S3	**23.42**	**40.80**	**42.82**

Table 2.2.4. DPF values in mm

Subject	**MLH**	**mLH**	**MP**
S1	**35.21**	**35.60**	**30.04**
S2	**33.61**	**33.65**	**33.82**
S3	**36.51**	**47.18**	**39.40**

Table 2.2.5. DEH values

Subject	**MLH**	**mLH**	**MP**
S1	**21.20**	**19.33**	**17.26**
S2	**15.01**	**19.97**	**25.74**
S3	**26.08**	**18.12**	**20.74**

Table 2.2.6. DEL values

Subject	MLH	mLH	MP
S1	15.21	8.41	6.80
S2	9.21	6.40	5.61
S3	7.20	10.00	6.01

Table 2.2.7. DECV values

Subject	MLH	mLH	MP
S1	22.00	15.81	18.00
S2	27.58	21.60	15.20
S3	19.62	16.40	10.00

Table 2.2.8. GP values (corresponding vertebra)

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

Table 2.2.9. 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

Table 2.2.10. AAMV values

Subject	MLH	mLH	MP
S1	34.40	37.09	28.99
S2	30.92	30.82	32.84
S3	32.80	44.09	37.31

Table 2.2.11. 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

Table 2.2.12. VFBS values

Subject	MLH	mLH	MP
S1	**45.869 cm^3**	**52.041 cm^3**	**cm^3**
S2			
S3	**37.424 cm^3**	**45.409 cm^3**	**71.186 cm^3**

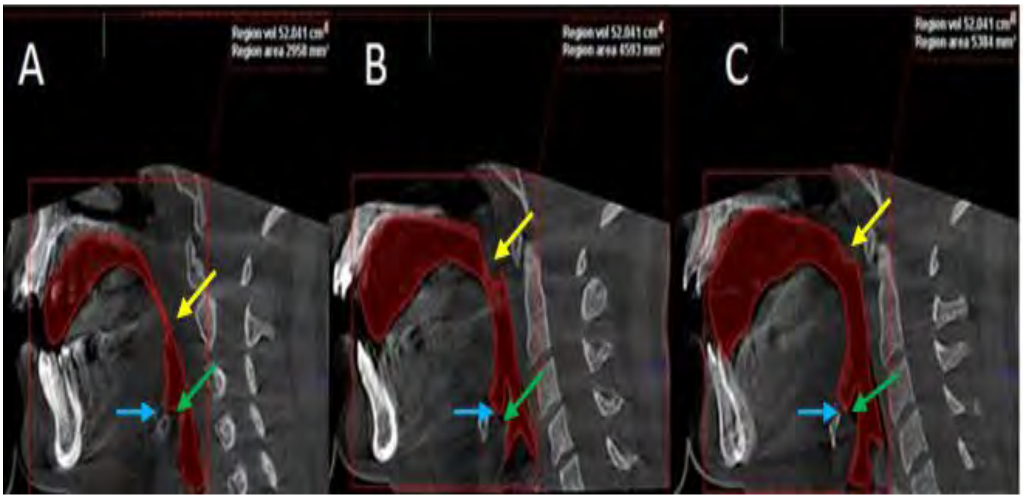

Figure 2.2.8. 3D reconstruction of buccopharyngealpavilion of subject S1; A - phonation with larynx in uppermost position, B - phonation with larynx in lowermost position and C - miming phonation. All 3D reconstructions are represented in sagital view. Yellow arrows mark the free edges of the palatine veil; green arrows mark the top of the epiglottic cartilage; light blue arrows mark the valleculae.

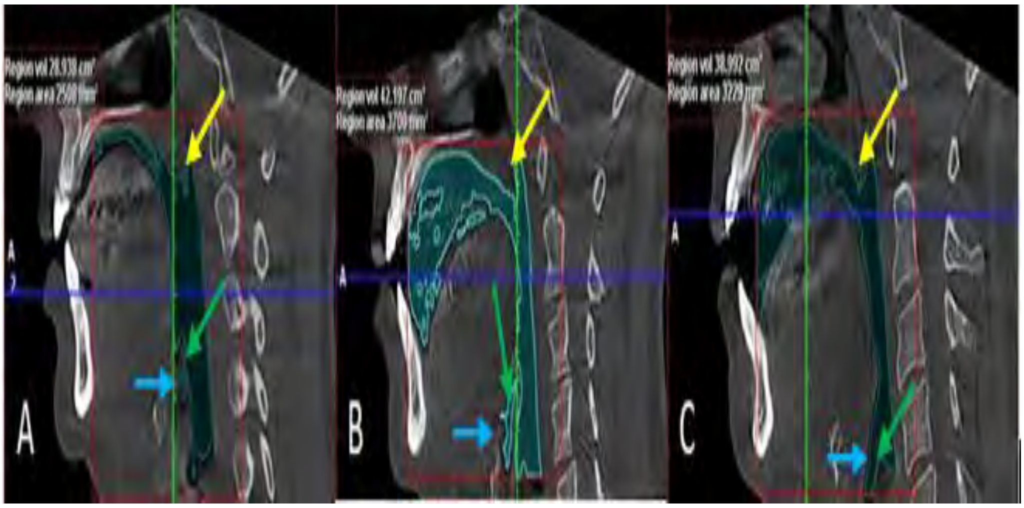

Figure 2.2.9. 3D reconstruction of buccopharyngeal pavilion of subject S2; A - phonation with larynx in uppermost position, B - phonation with larynx in lowermost position and C - miming phonation. All 3D reconstructions are represented in sagital view. Yellow arrows mark the free edges of the palatine veil; green arrows mark the top of the epiglottic cartilage; light blue arrows mark the valleculae.

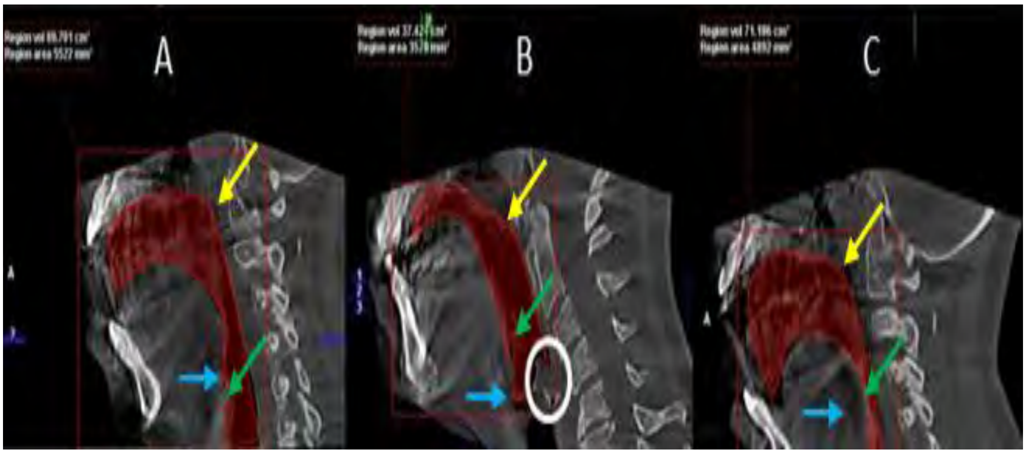

Figure 2.2.10. 3D reconstruction of buccopharyngeal pavilion forsubject S3; A - phonation with larynx in lowermost position, B - phonation with larynx in uppermost position and C - miming phonation; the white circle marks the anterior protrusion of the posterior pharyngeal wall due to the contraction of palatopharyngeusmuscles (Passavant's ridge). 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.

The 3D reconstructions performed on the three subjects showed significant differences in axial and coronal diameters for the measurements made in the same phonatory state, but also individual differences of these diameters for the measurements made in different phonatory states for the same subject. (Figures 2.2.8, 2.2.9, 2.2.10.).

The visualization of 3D reconstructions highlights the influence of the bucco-pharyngeal muscles (palatoglossus, palatopharyngeus, hyoglossus, genioglossus and longuscapitis muscles) on the volume and especially on the shape of the buccopharyngeal pavilion in sustained phonation (Fig. 2.2.11.).

After removing the artifacts and 3D printing of CBCT reconstructions, we covered them with a transparent gel coat polymer matrix, to better observe the buccopharyngeal pavilion.

2.2.5. Discussions

The opera voice has always attracted the interest of researchers from both the artistic and medical field, even though the latter may be more predominant. The complexity of the physiological processes and the functional adaptation of the morphology of the anatomical neural structures involved in opera voice formation are not yet fully understood.

Significant scientific data on the mechanisms underlying the opera voice formation are found in the artistic literature, although they are the results of certain medical studies.

The study of vocal training mechanisms is not deepened in medical faculties or in related medical specialties, as their main focus is the pathology of phoniatric organs - phoniatry.

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

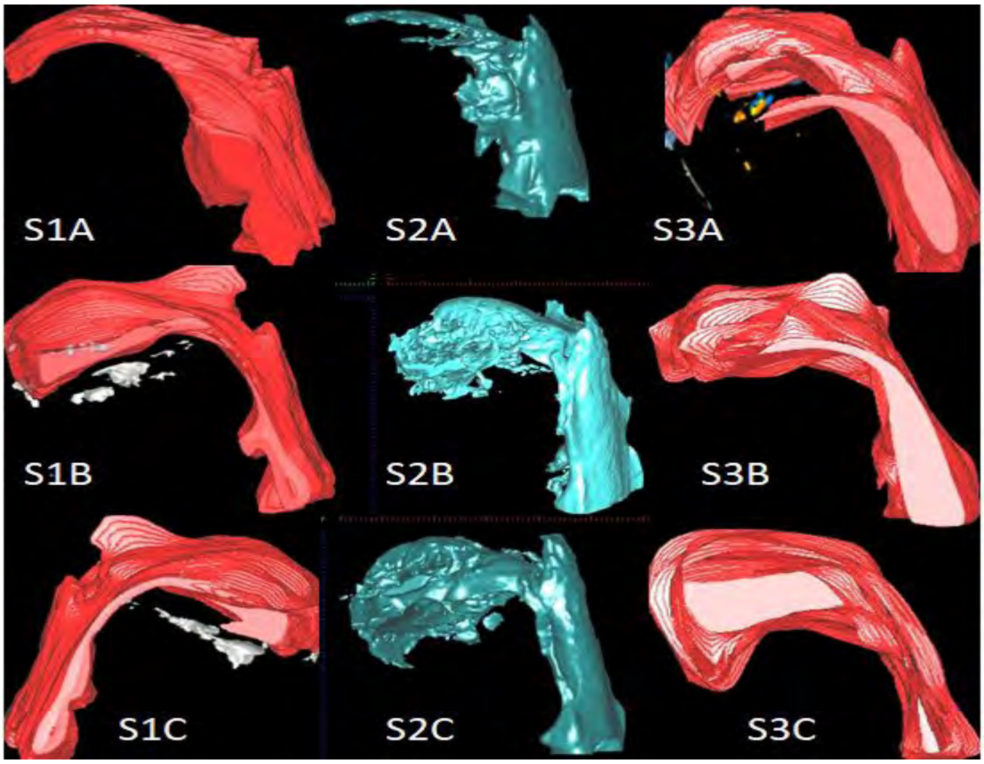

Figure 2.2.11. 3D reconstruction posterior lateral view; S1A, S1B and S1C - 3D reconstruction of S1 subject while phonating letters "I:", "Ə" and miming phonation; S2A, S2B and S2C - 3D reconstruction of S2 subject while phonating letters "I:", "Ə" and mimingphonation; S3A, S3B and S3C - 3D reconstruction of S3 subject while phonating letters "I:", "Ə" and miming phonation.

This study includes both a retrospective and a personal component. In the retrospective part we have analyzed the results achieved so far in the medical and artistic field (canto) on the neurological mechanisms underlying the process of opera voice formation. The study is under progress. The stages we have gone through so far are as follows: morphometric studies for the phonatory apparatus components made on anatomical specimens preserved in formaldehyde and MRI images. We are currently carrying out a morphometry and volumetry study on the pharyngo-buccal pavilion using the Cone-beam Computed Tomography (CBCT) technique. Our experience allows us to have an objective and pertinent approach to literature data, which,is often divergent.

This material is based on the knowledge gained so far about nervous control of breathing, dilators and constrictors muscles of the glottis and of the oropharyngeal phonation floor (palatine veil, palatine veil pillars, the hyoidian suspension apparatus and the base of the tongue). Thus, we can emphasize the existence of 3 levels (formants) of the phonation: inferior (infraglottic filter), middle that corresponds to the glottis and superior, oropharyngeal. These 3 levels of phonation work differently in ordinary individuals than in professional opera singers.

At the inferior vocal formant we studied the contribution of the infraglottic air column in the act of phonation.

At glottic level (the middle vocal formant), the muscles that are inserted into the vocal folds, thus allowing their mobilization have a movement controlled both by the recurrent laryngeal nerves and the superior laryngeal nerve. At the same time, the recurrent nerve presents a number of specific features, long-studied and well-known, which we will take in consideration.

The laryngeal sound is made at the level of middle vocal formantsand then suffers superimposed harmonic changes in the third formant (oropharyngeal).

The muscles that allow the descending of the palatine veil control the dimensions of the oropharyngeal isthmus and also the positioning of the pharynx to the epiglottis, which influences the quality of phonation.

The selection of the bibliographic sources is based on the chronological criteria of the findings, related to each of the five parts outlined above. In order to find the informative material, we consulted the following databases: PubMed, Clarivate Web of Science, and Scopus, using the following keywords: "voice training", or "larynx", or "canto", or "glottis", or "palatine veil", or "phonation", or "laryngeal nerves". We also found some of the important informations in specialized books.

2.2.5.1. ***The role of the infraglottic air pressure in phonation***

The vocal cords vibrate due to the contraction of the muscular attachments, as it will be shown in the following subchapter. However, the infraglottic pressure of the air column acts as a vocal formant by myotatic stimulating of the baro- and tensioreceptors of the internal thyroarytenoid muscles. These receptors increase the excitability of these muscles, reacting similarly as the palatal and pharyngeal receptors described below (Husson and Djian 1952).

This real glottic sphincter determines the descending of free edge of the vocal cords as the glottis opens, due to the contraction of internal thyroarytenoid muscles (Kirikae, 1943).

2.2.5.2. ***Glottic muscles innervation; special properties of recurrent laryngeal nerves***

Until 1950, it was thought that the vocal muscles are formed by a set of fibers 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 with a " hair comb" appearance. 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 muscular system consists of: a posterior fascicle, called *posticus*, represented by posterior cricoarytenoid fascicle, from 3 interarytenoid fascicles and lateral cricoarytenoid fascicles, called *lateralis*. Interarytenoid fibers are inserted into the ligament that fixes the vocal ligaments by the inferior thyroid notch and are known as the aryteno vocal fascicle of the internal thyroarytenoid muscle. Other muscular fibers originating from the internal surfaces of the thyroid cartilage are inserted on the vocal ligament forming the thyro vocal fascicle of the same muscle (Husson, 1951).

In order for the vocal fold to vibrate and emit sounds, it must be found in the so-called phonatory position, that is, in tension. This is made by constriction of the glottis, caused by contraction of the *lateralis* and ary-arytenoid muscles. Contraction of the cricothyroid muscles occurs after glottic constriction, which, by tilting the larynx, produces the tension of the vocal folds.

All of these muscles are motor innervated by the recurrent laryngeal nerve, except for the cricothyroid that is controlled by the superior laryngeal nerve (Husson, 1951).

Recurrent action potentials and voice vibrations were recorded simultaneously in 1953, by applying in vivo electrodes on the recurrent nerve connected to a double-path cathode oscilloscope. It has been demonstrated that the recurrence activity precedes the sound emission by 7-8 ms, which means that these are motor centrifugal pulses that precede phonation (Moulunguet, 1954).

The basis of the explanation of sound formation is given by the myolastic 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 innervated 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, Laget 1953, Portmann and Robin 1955, Piquet and Decroix1956).

The difficulty of highlighting this phenomenon is due to the synchronicity between expiration and phonation that occurs and develops as a motor pattern since childhood. This was first observed and demonstrated in 1957 (Galli and de Quiros 1957).

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 thus moving the two vocal cords and producing a ”vibratory” phase of the glottal opening (Fabre, 1957).

The direct conclusion that derives from these scientific experiments is that the vocal muscles have individual motor units originating in the recurrent nerve, that rhythmically contracts at the frequency of the voice emitted. Their action potential is between 0.8-0.9 milliseconds. McKinney and Heymann sustain the neurochronaxic theory (Heymann 1954, McKinney 1994).

Recent research contradicts partially or totally the neurochronaxic theory. To date, myolastic/aerodynamic theory has more followers than the previous one or other theories about phonation (Rubin, 1960).

Another current theory is that of the non-linear source filter. It considers that some of the acoustic energy is reflected back on vocal vibrations (Zemlin 1997, Kenneth and Bozeman 2013). The theory argues that an instability in vibration modes can occur when harmonics pass through formants during changes in the sound height or vocals. Unlike most musical instruments, a stable harmonic spectrum is not achieved by adjusting to the resonance frequency of the vocal tract but rather by placing the harmonics in the favorable reactance regions. This allows the positive reinforcement of the harmonics by over- and infraglottic reactants, without the risk of instability (Titze 2008, Lucero et al., 2013). At this point it is known that these muscles are made of intrafusal and extrafusal muscle fibers (Sanders et al., 1998).

Some studies suggest that the vocal cords muscles in humans behave in a particular way when compated to the rest of the mammals and similar to extrinsic ocular muscles (Keene 1961, Sartore et al., 1987, Lukas et al., 1994, Ruskell 1999), i.e., they do not exhibit stretching reflexes or myotatic reflexes (Pedrosa, 1995). Instead, these muscle fibers develop a "spindle-like" motor complex, consisting of receptors and highly adaptive kinesthetic afferents (Billig et al.,1997, Carla et al., 2003, Hunter et al., 2004).

2.2.5.3. *Returned impedance*

Functional features of recurrent nerves in phonation allow healthy people to emit high or low sounds with a maximum frequency ranging from 360 pulses per second in bass voice to 620-700 pulses per second in tenors or sopranos (Husson, 1960). This frequency range which physiologically and theoretically can be reached by any person is called *the first voice register or chest register*. In this case, the impulses through the recurrent nerves propagate "in phase", "impulse after impulse".

For a person to be able to emit sounds with a higher frequency than the one characteristic of the refractory period of his recurrent nerves, he must pass to the *second voice register* or the *biphasic register*. To achieve this, the recurrent nerves of that person must be divided into two fascicles, a phenomenon called biphasis (Husson, 1960).

The recurrent nerve biphasis is not an individual property being initially demonstrated on the auditory nerve (Stevens and Davis 1936). On the recurrent nerve, this was first demonstrated in 1955 (Coraboeuf et al., 1956). Studies show that the recurrent nerve, starting from a certain upward stimulation frequency, can respond in two ways:

- in phase but with half dimmed amplitude
- conducting two stimuli at once.

The first response variation represents the second register of the voice, characterized by an increased frequency of contraction of the vocal muscle and a decrease of its amplitude.

The superior limit of contraction frequency of vocal muscles in this register is double the frequency in the first register and is called the *superior limit of the falset register (in men) or the head register (in women).*

There are professional singers who have been shown to be able to emit sounds even more acute, higher than the upper limit of their second register. In this situation the recurrent nerve is divided into three fascicles, which act by rotation at one-third of the phase between them. This finding characterizes *the third voice register*, also called three-phasic, small register or *flageolet* register.

Mado Robin, soprano of the Paris Opera, is recorded in the history of chant as the only soprano-sopranissima capable of rising her voice with a quartet above it in the IV register of the voice. This register is characterized by the four-phasing of the recurrent nerves (Husson, 1960).

Basically, the result of sound frequency increase and, implicitly, the accentuation of the voice is a phenomenon called vocal coverage (converted). This phenomenon allows the singer to follow the ascending line while the phonatory effort decreases, which is one of the most important protection mechanism of the laryngeal phonatory apparatus.

Vocal coverage began to be known and applied in artistic practice from the 18th century in France and Italy. Its basis is made by the elongation and detensioning of the vocal cords by the forward tilting of the larynx and thinning of the ventricular slit. At the same time, the following movements take place: lowering of the larynx in the intermediate position, forward projection of the base of the tongue and lifting of the palatine veil.

In our studies, by videolaringoscopy and CBCT, on students of the Iaşi Canto Faculty and on the soprano of the Romanian Iaşi Opera, we have highlighted the same sequence of events that take place during the so-called passage (the transition to singing with the covered voice): it begins with lowering of the larynx, simultaneous with the anterior projection of the base of the tongue, followed by enlargement of the oropharyngeal cavity by relaxation of palatopharyngeal muscles.

Theresponse of the superior voice formant (the pharyngo-buccal pavilion) to the emission of harmonic glottic vibrations and to the shift from one register to another is different.

Thus, it was experimentally proved that the trigeminal spinal (from medulla oblongata) nuclei, which also control the articulated language, discharge nerve impulses in chronological frequency from the pharyngo-buccal pavilion to the larynx, respectively vocal folds (Krmpotic, 1957).

The role of the superior vocal formant in phonation is a very important one, requiring a morpho-functional adaptation prior to sounding. This vocal formant behaves like an acoustic pavilion that opposes the sound and air loading (Rocard 1935, Rocard 1949). Thus-explained answer of the pharyngo-buccal pavilion to the passage of the soundtrack through it is called “the returned impedance” and determines the increase of the glottic opening time, the thickening of the vocal folds and the increase of the glottic flow.

Returned impedance to the larynx is another protection mechanism of the laryngeal phonatory apparatus which can be added to the returned impedance by the nasal pavilion.

In our studies mentioned above, we have noticed that the nasal pavilion is permeable especially to open vowels, while in the issuing of acute closed vowels, it is partially or totally obliterated by the palatine veil. In the case of professionals, the phenomenon of nasalisation is almost absent in first and second registers of voice. The phenomenon of nasalization occurs especially at low sounds. On the examined subjects, phonatory adaptation of the pharyngo-buccal pavilion in the first voice register at the time of passage to vocal cover is spontaneous in professional singers and is missing in subjects lacking canto training. At the same time, there is an awareness of it in first year students.

All of these considerations lead us to the conclusion that the canto exercises develop within 6-10 years rhythmic motor patterns at the pharyngo-buccal and glottic level.

Research in this field continues today, when it is widely accepted that phonation mechanisms in the opera voice are physical, reflexive vocal tract adjustments that allow the human voice to produce a wide range of frequencies. These mechanisms directly related to the notion of vocal registers represent an important vocal quality parameter (Shanshan et al., 2016, Everton and Carlos 2017).

2.2.5.4. *Phonation function and innervation of palatoglossus and palatopharyngeus muscles*

While studying the neural mechanisms involved in the realization of the singing voice, we consider of importance to discuss about the participation in phonation of the superior vocal formant musculature. The palatoglossal and palatopharyngeal muscles, by their contraction, alter the shape and thus the function of the vocal tract.

These muscles have synchronous action during phonation. In our study, both pairs of muscles contract with increased intensity as the singer picks up a sustained vowel to its maximum possible frequency. When the singer passes into the second vocal register, the muscles relax, almost simultaneously with the detensioning of the vocal cords.

The feeling of soreness and pain felt by most individuals as they approach that limit seams to be due to the contraction of these muscles.

If the relaxation of the vocal cords is given by the indirect action of returned impedance from the pavilion, the relaxation of the palatine veil pillars suggests being reflexive to the glottic one.

Regarding the palatoglossal muscle, there is a dispute between anatomists about its belonging to the extrinsic tongue muscle group, which 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 appears 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).

Moreover, a number of authors classify this muscle as belonging to the palatine vault and not to the tongue (Fehrenbach, 2016).

A very important aspect is related to the innervation of this muscle. Classical anatomy describes palatoglossal muscle as the only tongue muscle that is not innervated by the hypoglossal nerve (XII) but by the vague nerve (X) (Burt 1996, Moore et al., 2014).

Other authors consider that the muscle is innervated by the glossopharyngeal nerve (IX) (Snell, 2010).

Classical anatomy describes palatopharyngeal muscle as being innervated by cranial nerve IX (glossopharyngeal). The palatopharyngeal muscular fascicles have an inferior lateral direction to the 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.

Dr. 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.

Most authors who have studied this muscular formation claim that this ridge is too small, too slow, too inconsistent, and with a too short contraction to be of essentail importance in the normal speech mechanism (Calcan, 1957-1958).

Its contraction plays the role of occlusion in the communication between nasopharynx and oropharynx, together with the lifting of the soft palate. So, the contraction of the muscle is synchronous to the contraction of the levator veli palatini muscles.

On the other hand, the anterior displacement of the posterior wall of oropharynx during ~~the~~ phonation is accomplished by the co-participation of Passavant's crest, that is, by contraction of palatopharyngeal muscles. Recent studies show that the anterior displacement of the posterior wall of the oropharynx is also due to the forward displacement of the lateral walls of the oropharynx. This action is the result of isometric contraction of the longus capitis muscle (Yamawaki et al., 1994, Yamawaki et al., 1996, Yamawaki 1997).

The contraction of the longus capitis muscle causes mass pushing on posterior oropharyngeal wall, while the contraction of the palatopharyngeal muscles (Passavant's crest) determines the anterior bulging of the posterior oropharyngeal wall (Yamawaki, 2003).

The occlusion of the nasopharyngeal isthmus is universally accepted as part of the reflex mechanisms of the swallowing (Pitman, 2009).

The implication of this "palatopharyngeal sphincter" in artistic phonation is not yet fully understood.

In our personal CBCT study we noticed the anterior protrusion of the oropharynx's posterior wall only in the case of a professional soprano. The images captured on this subject during sustained phonation on the vowels "I" and "U", are emphasizing this aspect. Protrusion does not occur when the subject is examined by the same method in the same position but without producing phonation (just mimicking the phonation).

All of these findings lead us to the conclusion that, at least in the case of opera voice, there is a reflex arch between the spinal nuclei of the vagus nerve and those of the glossopharyngeal and hypoglossus nerves. Repositioning anteriorly the posterior wall of the oropharynx occurs primarily in second register of the soprano's voice, as a result of the controlled contraction of the palatopharyngeal muscles without complete occlusion of the palatopharyngeal isthmus. Simultaneouslythere is relaxation of the palatoglossal muscles together with the anterior movement of the base of the tongue. We called this a pharyngeal-recurrential reflex.

The projections of the opera singers` subjective sensitivities, ~~so~~ ignored or blamed by some authors, appear to take shape in a scientifically demonstrated form. Nine of such projection areas have been described.

The first three are the most important, corresponding to the soft palate and palatine veil. The nervous pathway of these reflexes starts with the interoceptive receptors of the palatal mucosa and the vibrations receptors of bony palate. The information gathered by these neurons' dentrites reaches Gasser's ganglion and then synapses with the pontine trigeminal neurons. The ascending pathway, which goes upward from these sensory neurons, crosses over and makes a relay into the thalamus, then splits in two: a fibers group enters into the lobe of the island and the second one in the primary somesthetic cortex. All of these fibers form the direct pathway of these sensitivities, to which the indirect one is added, through the reticulate matter of the cerebral trunk and the posterior hypothalamus. From this level, the indirect fibers reach the thalamus, being then distributed to the entire cortex through *the diffuse activator system of Moruzzi and Magoun* (Moruzzi and Magoun1945, Lhermitte

1998).

Jean Mauran (on his real name Jean Joseph Antoine), first-baritone of the Paris Opera, was among the firsts to analyze these palatinal regions (Husson, 1960).

It was demonstrated (Husson 1951, Husson 1960) by stimulating the palate with electrodes that a weak electric current applied in that area stimulates the homolateral vocal cord, while a strong ~~one~~ electric current stimulates the muscularity of the oropharyngeal pavilion. The same researchers have made the cocainisation of the soft palate, unilaterally, noticing a decrease in glottic and oropharyngeal muscular tonus (unilaterally as well), with appearance of dysphonia.

Other researchers (Husson, 1960) cooled the first and second palatinal regions, unilaterally, and found the occurrence of hypotonia of the homolateral vocal cord.

The ascending pathways of these reflexes can reach the heterolateral ambiguous nucleus, and then synapse with the motor nuclei of the cranial nerves V, VII, IX, XI and XII. Thus, stimulation of inferior and superior salivary nuclei and of the cardio-pneumo-enteric centers occurs. These stimuli activate the origin neurons of the recurrent fibers of the vagus nerves, by reflex activation of these nerves in terms of their biphasic or even triphasic status.

2.2.5.5. *Brain control of the opera voice*.

The cortical control of vocal muscle contraction and, implicitly, of vocal folds vibrations is different in professional opera singers. They seem to develop a rhythmic motor pattern, highlighted by the experimentally demonstrated fact that the recurrent motor nuclei from medulla oblongata are in a state of autorithmicity. This explains the speed and ease with which the professional singers activate these nuclei (Husson 1950, Monnier 1951, Gaillard 1956).

The pontine and medulla oblongata reticulate matter has direct links with all phonation motoneurons, having a role in generating complete vocal models. This region receives impulses from two different ways of voice control:

- the first one contains the anterior cingulate cortex and the periaqueductal gray matter, both causing vocalization to electrical or pharmacological stimulation;
- the second one includes the primary motor cortex and two subcortical circuits composed of putamen, globus pallidus, gray pontine matter and cerebellum, that send modified motor programs through the ventrolateral thalamus to the main motor area; the electrical stimulation of the ventral portion of the main motor area produces vocalization and contractions of the muscles involved in the phonation.

I. Scale in 3-level of voice control

The reticulated formation is the most inferior level in which complete vocal patterns are formed. The anterior cingulated cortex and periaqueductal area are associated with voluntary initiation and emotional control of vocalization. The main motor area (M1) is associated with the generation of aquired phonation such as speech and singing, having also direct connections to motor neurons involved in speech, which allows the ability to form innate sounds even after M1 damage (Zarate, 2013).

II. Somatosensitive processing

Information from the somatic receptors is transmitted via the glossopharyngeal and vagal nerves through the gracilis, solitary, spinal nuclei of the trigeminal nerve and the medial lemniscus to the ventral posterior thalamic nucleus and from here to the primary and secondary sensory cortex (S1 and S2) as well as to the isle lobe. They reach especially to the ventral portion of S1 adjacent to the M1's phonatory region in order to control the movements initiated by it, contributing to breathing control during phonation.

III. Auditory processing

The information needed to control the tone is transmitted and processed along the auditory pathway composed of the cochlear nucleus, the lateral lemniscus, the inferior colliculus, and the medial geniculate nucleus of the thalamus. The tone information is specifically processed by an area predominantly located in the right hemisphere, on the lateral side of the Heschl's gyrus, which places the different frequencies in a hierarchical order for voice control (Rosslau et al., 2016).

Frequency perception also includes areas of the superior temporal gyrus: planum polare and planum temporale. Musical phrases that spread over a range of frequencies also determine a response in the intraparietal sulcus.

The vocal tone is also processed along the superior temporal sulcus, thus having a role in recognizing heard voices.

IV. Integration substrate of sensory feedback in speech motor control

The periaqueductal matter and the anterior cingulated cortex receive both somatosensitive information (from the gracilis, solitary, spinalis nuclei) and auditory (from the inferior colliculus and the lateral lemniscus) that contribute to the realization of the phonation in relation to external stimuli and to the sensory control of phonation. This is demonstrated by keeping the Lombard reflex in, which indicates the role in governing the auditory-motor reflexes.

The anterior cingulated cortex directly receives data from the somesthetic area 2 and areas from sulcus and superior temporal gyrus and indirectly receives data from the somesthetic area 1 and the association auditory areas through the isle lobe. Due to the fact that it receives both auditory and somatosensitive pulses, the anterior cingulated cortex can be considered an area of integration, especially in its anterior part (Perkell, 2012) .

V. Effects of singing exercises on the somatosensitive control of singing voice

It is known that musicians have a greater capacity to differentiate between tones (Bosnyak, 2004) and have a more precise control of vocal motor function in the absence of auditory feedback; the trainees are more reliant on somatosensitive feedback (Rosslau et al., 2016), as demonstrated by studies using fMRI (Perkell, 2012) and magnetoencephalogram (MEG).

Studies using topical anesthesia show the role of the isle lobe in this type of control, a decreased of the isle lobe (Ackermann and Riecker 2004, Chen et al., 2012) being more pronounced in professionals than nonprofessionals, even though the first still retains a more precise phonatory capacity.

Other studies on tonality control reveal the involvement of dorsal premotor cortex and intraparietal sulcus (Foster and Zatorre 2010) in nonprofessionals in the process of learning, while musicians use predominantly posterior areas of the superior temporal sulcus and anterior cingulated cortex to perform the same sequences (Simonyan and Horwitz 2011).

Up to date, 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 perform in singing, or if these skills are acquired.

The phonation in canto voice is different from the usual and even from 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.

2.2.5.6. *Morpho-radiological approach of the opera voice*

2.2.5.6.1. *Anatomical study*

The purpose of this study is to highlight the role of the orthograph of the pharyngeal-oral pavilion in the formation of theatrical voice through morphometry and volumetry achieved by 3D assessment of the images acquired by with the CBCT technique. We have made the three-dimensional, physical and digital experimental evaluation of the morfofunctional changes occurring at the level of the bucophorian pavilion in the opera and theater voice, to establish anatomical and physiological particularities of this floor of the vocal tract, necessary for the artists in this field.

The results obtained open the way for research in the neurological sphere of phonology, laryngology and medical technique. By comparing the results obtained for individual sex and vocal training, we highlight the anatomical formations and the most demanding segments of the interest region (hot spots) and we aim to create a digital model of the space of interest, in view of quantification of artistic vocal abilities in terms of morpho-functional parameters.

Interdisciplinarity is another important feature of this study, as we partnered with experts from different fields - anatomy, otorhinolaryngology, internal medicine, canto, science, materials and dynamics of fluids. Moreover, the results concerned clinical and applied anatomy, canto and phoniatric fields as well as art industry. The value of the results is the summation and completion of current knowledge and research that focuses on the study of the glottic floor of the vocal tract and gives the possibility of defining the morpho-functional aspect of the bucophorian pavilion as voice forming.

"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 cords and vocal pavilon. We have found that these anatomical parameters encode the perceptual vocal identity (male, female, child) and show that brain areas responding to human speech also encode vocal identity " (Assaneo et al., 2016).

Our data collected through direct laryngeal morphometry on dissection specimans are very close to those in the literature (Kaur et al., 2014, Joshi et al., 2015, Patel et al., 2016) and confirms the substrate of the fundamental differences between the two genders regarding the particularities of phonation in adults.

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

Measurements performed on MRI images are also very close to those obtained by direct measurements and consistent with literature data (Lingala et al., 2016). However, we have not found in the literature another study that correlated the values obtained from these measurements (direct and imaging) nor did we find a study that would have produced statistical formulas based on measurements. 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.

2.2.5.6.2. ***Radiologic study***

In the literature we have not found data (investigating sources as Clarivate, PubMed, Scopus, Index Copernicus or specialty books) about the CBCT exploration of canto singers (sopranos in this case). This makes us claim/conclude that we are the first to realize this procedure.

We conducted this study in order to reveal the morphometry and volumetry of this space under different phonation positions.

We performed the interpretation of the obtained data for each subject in part, as well as comparatively in the same phonatory positions.

DPG value

In S1 we note that this distance decreases in phonation with the larynx in superior position and increases in phonation with the larynx in inferior position, compared to the value measured in the mimed phonation, ranging between minus 17.14% and plus 14.3%. This does not happen in subjects S2 and S3, where DPG value decreases in both situations (13.9% and 18.4% - S2 respectively 45.3% and 4.7% - S3).

We notice in S3 a significantly increased variation of 40.6% of DPG in the two phonatory positions, close to the 31.44% value found in subject S1. *However, in the case of experienced soprano (S3) this variation is totally negative.*

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

DPF value

The values of this measurement are very different from the previous ones, so that in the case of S2 they are smaller in both phonatory situations than in the mimed phonation but slightly insignificant (minus 0.62% and minus 2.9%).

The both values are higher in phonation in subject S1: plus 4.12% and 18.51%.

In S3, the values in phonation are diametrically opposed to the control value (phonatory repose) - less 7.34% and plus 19.75%.

After comparing the DPG and DPF values of S1, it is noted that the oropharyngeal isthmus narrows from the posterior to the anterior in the phonatory state I and opens anteriorly in the phonatory state II.

In the same way, in the case of S3, the opening of the isthmus will be backwards to S1: the posterior diameter decreases by 37.96% more than the anterior one in the phonatorystate I. In the phonatory state II, both values decrease compare to the control, but the proportion is reversed in the favor of the anterior opening with plus 15.05%.

S2 has plus values of 13.28% and 15.5% in the two phonatory states, in the favor of the anterior opening of the isthmus.

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 diametrically different (posteriorly opened in the phonatory state I and anteriorly opened in the phonatory state II).

DEH value

This measurement was performed in order to evaluate the position of the epiglotte to the hyoid bone (mobile point), which indirectly reveals the interaction of the hyoidian suspensory muscular apparatus in the act of phonation.

S1 presents DEH values higher than the reference value of the mimed phonation by 22.83% in the phonatory state I and 12% in the phonatory state II respectively.

In S2, the values are lower than the control value in both phonatory states: 41.69% and 22.43%, respectively.

S3 presents in phonatory state I a lower DEF value of 25.75% compared to the control value and in the phonatory state II a value increased with 12.63%.

Interpretation of these results reveals that S1 produces an anterior protrusion of the oral floor in both the phonatory states, S2 a retropulsion, and S3 produces a retropulsion in the state I and a protrusion in the state II.

DEL value

The DEL values are increased compared to the control value in all the three subjects, as follows: S1 with 123.68 and 23.6, S2 with 64.17 and 14.08 and S3 with 19.80 and 66.39, in the phonatory state I and in II respectively.

Absolutely particular are the values of this measurement for the experienced soprano, where the increases are inversely relative to the other two subjects - the DEL value increases much more in the phonatory state II than in I. This means that the first two subjects produce a greater anterior protrusion of the tongue in the phonatory state I than in II and S3 behaves diametrically opposed.

DECV value

This measurement reveals that the subject S1 has an increase of 22.22% compared to the reference value in the phonatory state I and a decrease of 12.17% in the phonatory state II. In S2 and S3, these values increase in both phonatory states by 81.45% and 42.11% respectively by 96.20% and 64.00%.

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

GP value

The skeletotopic projection of the glottis in S1 reveals that it is more elevated in the phonatory state I and more lowered in phonatory state II.

S2 presents the same glottic projection in the phonatory state I as in the reference situation and a lower one in phonatory state II.

S3 presents a glottic projection somewhat similar to S1 (elevated in the phonatory state I and lowered in phonatory state II), but the glottic elevation in phonatory state is located above with an extra vertebra.

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

ASMV value

For this measurement, we recorded higher values at all subjects in both phonatory states.

S1 and S2 show a significant increase in ASMV in the phonatory state I and much lower in the phonatory state II (48.43% and 8.15% in S1, 76.53% and 23.23% in S2).

S3 shows slightly increases in the sagittal diameter of the glottis (11.98% and 20.37%), inversely proportioned with the other 2 subjects: ASMV growth values in the phonatory state II are approximately twice as high as in the first.

The experienced soprano soloist achieves an amplification of the length of valleculae in the phonatory state II than in phonatory state I, contrary to the other two subjects.

AAMV value

Measurement of the transversal diameter of the valleculae has a completely different presentation in the three subjects.

S1 presents an increase in this diameter in the both phonatory states by 18.66% and 27.94% respectively.

S2 presents a decrease of this value in the both phonatory states, with 5.85% and 6.15% respectively.

S3 presents a decrease of the AAMV value by 12.09% in the phonatory state I and its increase by 18.17% in the phonatory state II.

Analysis of ASMV and AAMV measurements indirectly indicates the volumetric changes occurring at the level of the valleculae in the singing voice. To this respect, S1 presents an increase of sagittal and transversal diameters in both the phonatory states. S2 reveals increases in sagittal diameters in the both phonatory states but partially compensated by decreases in transversal ones.

In the case of the experienced soprano, the increase of the sagittal diameter in the 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 the both phonatory states and in S3 this happens only in the phonatory state II.

PVP and VPP values

The analysis of the position of the palatine veil and the skeletotopic projection of its free edge reveals us in the case of the S1 that it is almost horizontal in the phonatory state I and in the mimed phonation and it is obliquely descended in the phonatory state II.

S2 presents a horizontal palatine veil in the phonatory state I and an oblique one in the other two situations. In the phonatory state II it is lowered than in the control state.

S3 presents a horizontal position of the palatine veil in the three situations but slightly lowered in the phonatory state I.

After analyzing these values, we can conclude that S1 and S2 are nasal in the phonatory state II while the experienced soprano in none of the situations.

Volumetric values

In the case of the volumetric measurements of the buccopharyngeal pavilion, the comparative results show that at for S1 the values remain identically in the three situations, whereas at S2 and S3 they vary. For the last two subjects the smallest volume corresponds to the phonatory state I and the highest corresponds to the mimed phonation.

Specific to the experienced soprano is that the difference in volume between the phonatory state II and mimed state is very small (20.86%) compared to the phonatory state I when it decreases to almost a half (47.43%).

Morphometry and volumetry of the buccopharyngeal pavilion is different in the 3 subjects. In the case of professional canto voice, the appearance of different parts of the superior vocal formant suffers particular functional changes. In phonation with elevated larynx, the oropharyngeal isthmus is strongly open anteriorly and reduced posteriorly, with the retropulsion of the oral floor, slightly anterior protrusion of the tongue and epiglottis, elevated laryngeal position, constant vallecular volume and horizontal palatine veil. In phonation with lowered larynx, it has an oropharyngeal isthmus slightly opened anteriorly and strongly opened posteriorly, a strong anterior protrusion of the oral floor, epiglottis, and base of the tongue, with the larynx in inferior position to the repose one, strong opening of the vallecular spaces, and a horizontal palatine veil. We consider that CBCT is a new and extremely useful method in appreciating the anatomical imagistic and function of the superior vocal formant.

The data recorded in this study is the starting point for similar explorations of the other types of singing voices, from the baritone to the sopranissime (Rodrigues, 2018).

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

Using CBCT in exploring the superior vocal formant is a new and complex method. Choosing the right imaging protocol that encompasses the entire explored area and fixed landmarks ~~is~~ was the key to this functional radiological investigation. The ability to precisely measure soft structures and spaces between them with minimal exposure to X-rays make CBCT a highly performing and useful exploration. Making 3D reconstructions and their recording brings for the first time information about the changes that take place during phonation at the oropharyngeal pavilion. Our pilot study demonstrates the usefulness of CBCT in the study of superior phonatory floors, opening the way for future studies on statistically significant lots of subjects. The practical applicability of our study is especially in the study of functional and clinical anatomy of morphology and volumetry of the oropharyngeal space.

2.3. ANATOMICAL SUBSTRATE OF NEOVASCULOGENESIS PROCESSES IN COLORECTAL CANCERS

2.3.1. State of the art

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 one of the worse? prognosis because it is characterized by a high local recurrence rate and a high metastasis presence at the moment of diagnosis (Folkman, 1971). The fundamental principle of the curative treatment of these tumors remains surgery, but even that has undergone major changes over time. Nowdays, a multidisciplinarity approach is mainstay and its main purpose is individualizing the therapeutic strategy, according to patient and tumor characteristics (Adam 1987, Liao et al., 2002).

Quantitative and qualitative assessment of prognostic factors in patients has particular importance, both preoperative, but most especially postoperative. The following paramters are of primary importance: 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. Weighing all factors, we have to take the best decision regarding the necessity of pre and postoperative neoadjuvant therapy as well as the decision on the appropriate surgical techniques (Bosari et al., 1992, Hall et al., 1992, McDougall et al., 2002).

Colorectal neoplasia has an increasing incidence among the population, and this fact compels in achieving an early diagnosis and treatment protocols. High-resolution magnetic resonance brings relevant data on the normal anatomical aspects of the rectum as well as on the possible pathological changes. The magnetic resonance imaging (MRI) investigations, in our study, bring us important information about the location and extent of the tumor, if it affects the mesorectum, its relations with the peritoneum, with surrounding fascia and organs, highlighting the rectal arteries and the neoformation vessels.

We evaluated the degree of extramural invasion of the blood vessels [extramural vascular invasion (EMVI) score] and thus, the impact of this determination in diagnosis, treatment and prognosis of colorectal neoplasia setting. According to American Joint Committee on Cancer (AJCC), the most common method in this respect is the one that takes into account the extent of the tumor, the invasion or not of the lymph nodes and the presence of metastases – TNM staging.

The EMVI score is another method used for staging cancer, besides TNM. It defines the presence of malignant cells in the blood vessels, outside its own vascular tunic, near the tumor. This score is long recognized as an independent predictor for local and systemic recurrence as well as for long-term survival (Milliaris et al., 1995, Herbst et al., 1998, Sabo et al., 2001).

Also, this score is used as an indicator for oncological therapy. The accuracy of approximately 52% of the EMVI score can be greatly increased by correlating it to MRI, as in the early stages of cancer it is difficult to assess whether a vascular structure is visualized or not. The accuracy increases in advanced stages where vascular structures are invaded, as they have the same native density as the tumor and ~~it~~ captures intramural contrast (Lidgren et al., 2005).

The complex processes of angiogenesis as the outgrowth of new vessels from a pre-existing 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. It is also a key player during the initiation and progressive growth of most types of solid tumors and metastasis (Sagar and Pemberton 1996, Salerno et al., 2006), tumor cells and blood vessels forming together a highly integrated ecosystem. Blood is a complex fluid, the rheological properties which lead to interesting feedback mechanisms during perfusion. Shear stres generated within the capillary bed by the flowing blood, strongly influence vessel adaptation and network remodeling (Cervantes et al., 2007).

Shear stress is then affected by blood viscosity which depends on a non-uniform distribution of the 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. We now know of a large number of components that induce angiogenesis.

The transition from the dormant avascular state to the vascular state, where the tumor possesses the ability to invade the surrounding tissue. Metastasis depends upon the ability to induce new blood vessels from the surrounding tissue to sprout towards and gradually penetrate 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 (Torkzad et al., 2010). For these reasons, the microvascular intratumoral density was suggested as a criterion for prognosis in different types and locations of cancer, being used in evaluating the evolving of the breast cancer (Bokey et al., 1997, Smith et al., 2008, Ayuso et al., 2010), renal cancer (Horn et al., 1991, Harrison et al., 1994, Smith et al., 2008), rectal cancer (MERCURY Study Group, 2006), bladder (MERCURY Study Group 2007, Slater et al., 2007) or prostate cancer (Sebag-Montefiori et al., 2009, Sauer et al., 2012, Al-Sukhni et al., 2013).

There were described several methods for tumor vessels identification based on the ability of endothelial cells of the vascular tissue to release antigens. These can serve as markers on tissue included in paraffin, and CD34 is considered to be the best marker for neovascularization (Taylor et al.,, 2008). Regarding the above considerations, we applied three different methods of neoangiogenesis evaluation on angiography images of resection specimen. We noted from the special literature data that this type of measures have not been previously tried in cancers in this location.

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.

2.3.2. Material and methods

2.3.2.1. *Angiographic study*

This paper is part of our studies on the vasculature of colorectal carcinomas quantifying high-density neovascular area using three different methods. Each method applied by default topographies of moderately differentiated rectal carcinoma (upper, middle and lower). We have to mention that all these cases were TNM staging IIIA and the patient ages were quite similar.

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

2.3.2.2. *MRI and CT studies*

This study was conducted on a group of 10 patients, colonoscopicaly diagnosed with rectal cancer, who underwent preoperative contrast MRI to establish tumor localization and resection possibilities. 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, seven were male, aged 46–57 years and three females aged 55–62 years. Of these, five 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 stage and two with lower rectal cancer, T1 and T2 stages.

We correlated the results obtained for each patient with the imaging aspects of the perilesional neoangiogenesis processes. We have used computer tomography (CT), nuclear magnetic resonance imaging (MRI) and 3D reconstruction techniques in the pelvis. These methods revealed changes in number, appearance and caliber of intra- and extra-colorectal vessels at the level area of the malignant formation. The 11 cases studied were evaluated by colonoscopy, CT and MRI.

2.3.3. Results

2.3.3.1. *Angiographic study*

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.

We have applied the Monte-Carlo method on the same areas of maximum neovascularization, to calculate surface area. The results are very close to those obtained by triangulation method: on the superior rectal neoplasm we obtained a value of 530 866.2526 mm2 (Fig. 2.3.1.), for the middle rectal neoplasm the value is 334 281.198 mm2 (Fig. 2.3.2) and for inferior rectal neoplasm is 116 990.2554 mm2 (Fig. 2.3.3.).

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 were significant and similar to those obtained by the triangulation method.

The other two types of rectal carcinomas show different aspects: in superior rectal carcinomas, we did not find on our images a zone without neoformation vessels. In inferior rectal carcinomas we encountered the oposite situation.

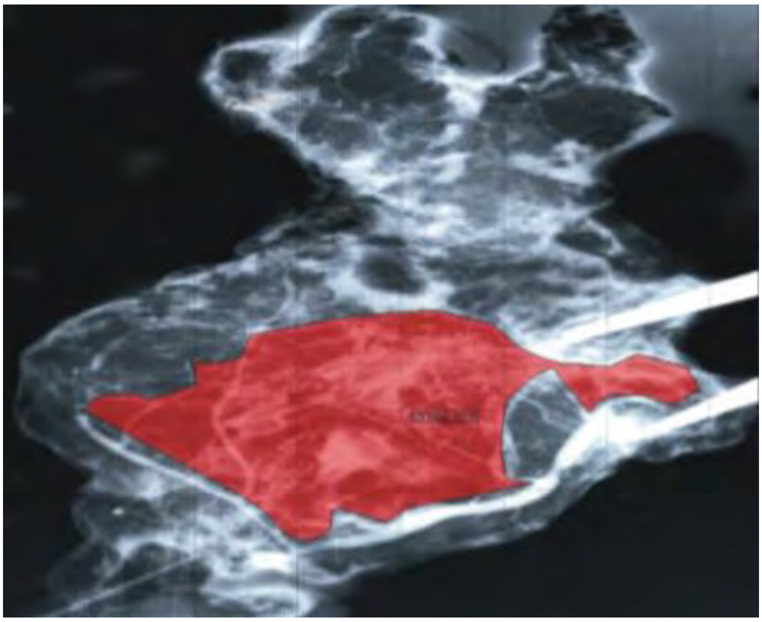

Figure 2.3.1. Monte-Carlo methods applied on superior rectal carcinoma

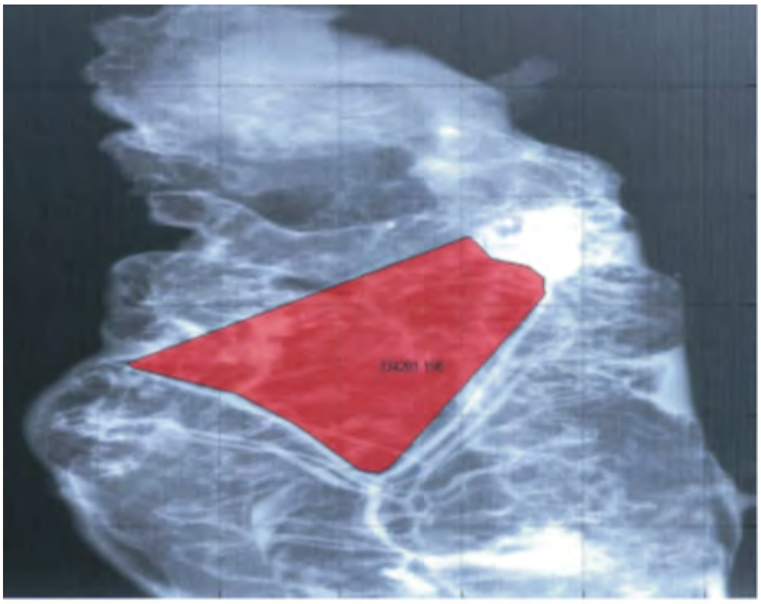

Figure 2.3.2. Monte-Carlo methods applied on middle rectal carcinoma

2.3.3.2. *MRI and CT studies*

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 (Fig. 2.3.4.). The presence of tumoral signal in the vascular structures is a landmark for tumoral presence (Fig. 2.3.5.). Affected vessels increase their volume and their inside signal is represented in medium gray.

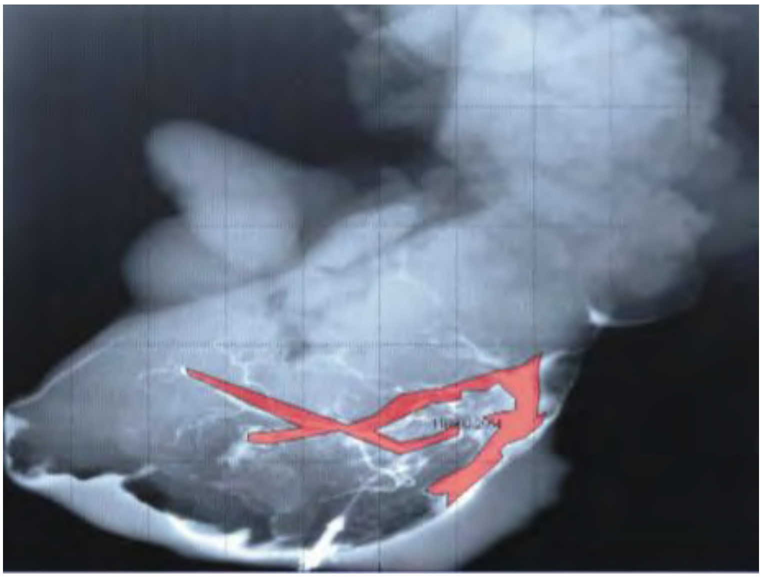

Fig. 2.3.3. Monte-Carlo methods applied on inferior rectal carcinoma

Extension of tumor inside the vessels causes a smooth, nodular or an irregular appearance of vessels (Fig. 2.3.6.). In none of the 15 cases of MRI investigated patients who were not found having rectal neoplasia could we amend vascular corresponding EMVI score. Moreover, in these patients we have not discovered any polyps, diverticula, or autoimmune diseases - benign formations, which could be classified as preneoplasic condition.

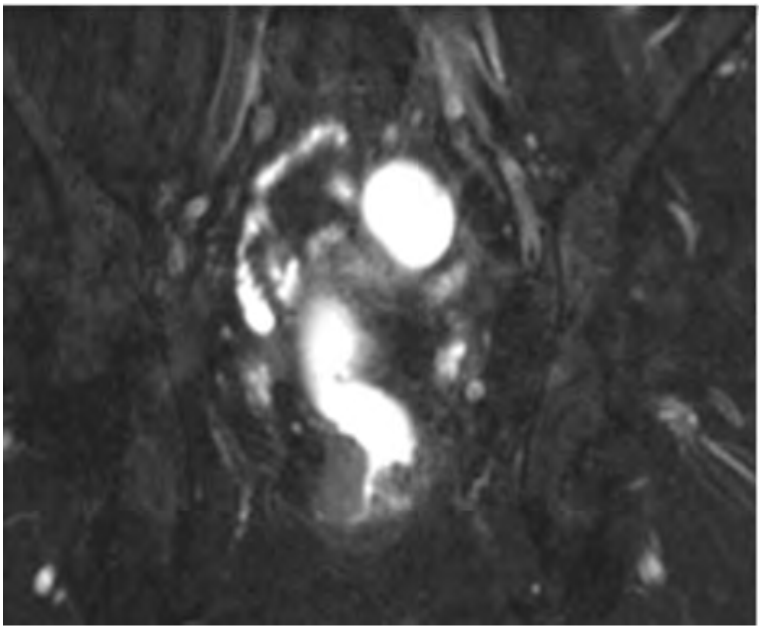

Figure 2.3.4. Pseudo-inferior rectal artery stenosis in the left, with similar changes on the contralateral inferior rectal artery, both intramural nodule type, specific to stage 3 EMVI

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 craniocaudal 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.

The graphical representation of the reported changes highlights the significant increase in tumor microvascular density with increasing grading, inversely proportional to the degree of differentiation.

Generally, in metastatic tumors, microvascular density is greater than in nonmetastatic tumors. In addition, there are large variations in vascular density between different areas of the same tumor in poorly differentiated forms, whereas in well differentiated carcinomas the topographic location is more constant. In the central, perinecrotic and extratumoral areas, microvascular densities are similar.

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/mm2 tumor exhibited the same change of magnitude, from 670.54/mm^2 to 1053.84/mm2 and 2250.69/mm^2 tumor respectively (Table 2.3.1.).

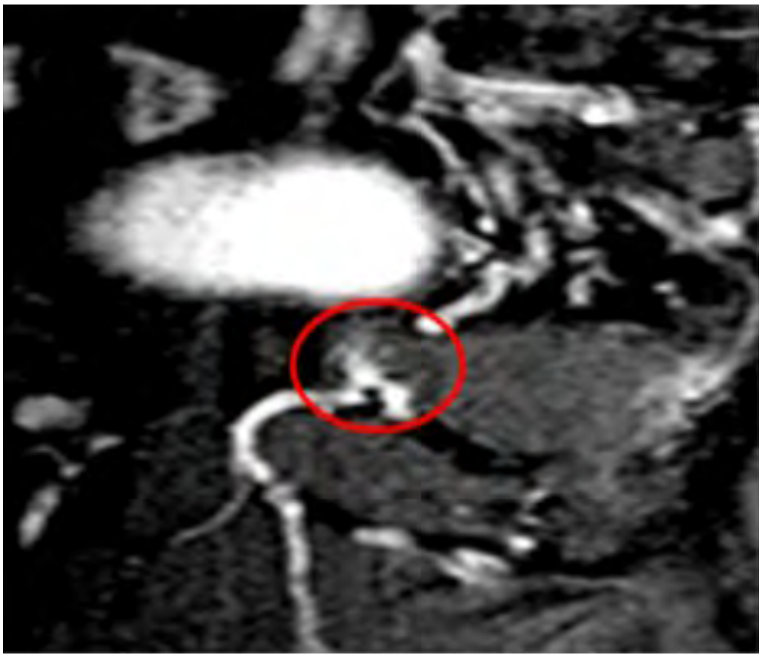

Figure 2.3.5. Left middle rectal vein in sagittal section, in a middle rectal cancer with intraparietal invasion – stage 4 EMVI

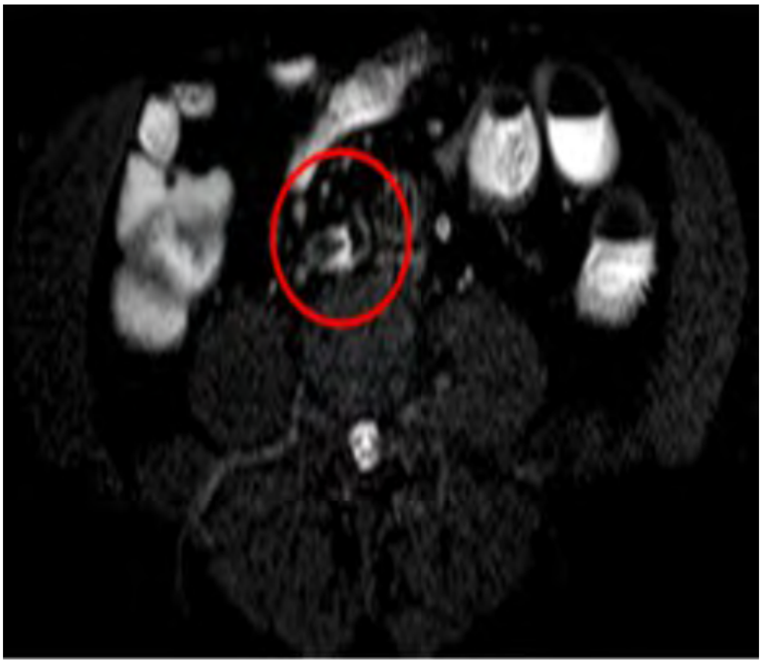

Figure 2.3.6. Neoformation vessels on the right inferior rectal artery, in MRI with multicenter condensing processes, characteristic for stage 3 EMVI

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. The degree of extramural vascular invasion on MRI images, ranges from minimal to extended, following four criteria: tumor edge, tumor location, vessel size and its appearance. The lowest score, 0, correlates with the absence of any suggestive characteristic for extramural vascular invasion. The maximum score, 4, correlates with the most obvious features of vascular tortuosity, nodular and obstructive appearance.

Table 2.3.1. Tumor type, correlated with the density of microvessels on mm^2

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

2.3.4. Discussions

Malignant pathology that affects the perianal region includes rectal cancer as a subtype of colorectal cancer (CRC) and anal cancer. Rectal cancer has an incidence of 35% of the total incidence of colorectal cancer, ie 15-25/100,000 per year. High mortality is recorded among males, with women being less affected, 4-10/100,000 per year. Most colorectal cancers develop from adenomatous polyps or adenomas. In America, the incidence between 20-24 years is 0.85 out of 100,000 and at 45-49 years, the incidence is 28.8 per 100,000, so there is an increase in incidence among young people and a decrease among the elderly (Poynter et al., 2013).

Following the 1987-2006 surveillance of colorectal cancers among 20- to 49-year-olds, there was an increase in incidence (10.7 per 100,000 in 1988 and 17.9 per 100,000 in 2006) (Edwards et al., 2010). In the study by You Y.N. (You et al., 2012), of the 70% of Cancer Cases in the U.S. there is an increase in 1998 incidence compared to 2007 of colorectal cancers of 1.1% to 3.1%.

CRC mortality in the 2005-2009 period ranges from 0.2 to 7.7 with an increase among the young population aged 20-24 (Siegel et al., 2009). In the study made by Huw (Huw et al., 2017) between 2002 and 2013, which included 98 patients with colorectal cancer under the age of 50 years of which 49 diagnosed with rectal cancer and 49 with colon cancer, it was noted that most presented with stage 2 or stage 3 CRC. There was no evidence of advanced disease when compared to the group of over 50 years. We can conclude that the incidence is increased in young people under 50, but with less advanced forms.

For cancer staging, the TNM system is used under its several versions. ESMO recommends version 5 of 1997 versus version 6 (2002) or 7 (2010) due to the variability of Stage II and III definition. Thus, stage 0 (Tis N0 M0) is in situ carcinoma, at intraepithelial level or invasion in the proper lamina with submucosal extension (Stage I-T1 N0 M0) and muscularis propria (Stage I-T2 N0 M0), Stage IIA with extension in subserosa/perirectal tissue depending on extension size subclassifiable T3a (<1mm), T3b T3c (> 15mm), T3d (> 15mm), Stage IIB (T4N0M0) with perforation in visceral peritoneum (b) or invasion in other organs (a), Stage IIIA (T1-2N1 M0) with 1-3 regional ganglia involved, IIIB (T3-4 N1 M0) with 1-3 regional ganglia involved, Stage IIIC (T1-4 N2 M0) with ≥4 regional ganglia involved, and Stage IV (T1-4 N1 -2 M1) with distant metastases (Glynne et al., 2017).

Squamous cell cancer is a rare pathology with an incidence of 1% -2% of digestive cancers and 2-4% of cancers of the colon, rectum and anus. Annually, the incidence is 1 case per 100,000 with a tendency for female growth. In Europe, about 2,000 men and 2,300 women are diagnosed with anal cancer each year. The National Cancer Institute estimates that in 2013, approximately 7,000 cases were diagnosed in the United States. The average age is 60 years (NCI, 2013).

Squamous cancer is closely correlated with HPV (human papillomavirus) infection, being the determinat factor in 80-85% of cases. HPV produces skin and mucosal infection in the mouth, anus, penis, and female genitalia. Of the multiple serotypes with oncogenic risk are 16, 18, 31, 33, and 35, and it associate with anal and cervical cancer. From the pathophysiological point of view, the precursor lesion is considered to be an anal squamous intra-epithelial lesion (AIN), which is, in turn, classified in low grade intraepithelial lesion (LSIL) with AIN I and HSIL (high grade intraepithelial lesion) comprising AIN II and III (Glynne et al., 2014).

Progression from AIN I to AIN III to malignancy is rare, and generally appears in HIV + immunosuppressed individuals with low CD4 cell counts (T-lymphocytes, monocytes, macrophages, dendritic cells) and HPV + lesions. Histologically, anal-skin tumors that are well differentiated appear in men, and those low differentiated appear in women. Histological subclassification in bazaloid, tranzitional, spheroidal and cloacogenic are not important in the management of pathology.

The biology and prognosis of keratinizing and non-keratinizing tumors is similar, although a variant of anal epidermoid cancer, verrucous carcinoma, which presents as a giant tumor or Buschke-Lowenstein tumors, has a better prognosis after surgical treatment (Glynne et al., 2014).

Anatomically, the limits of the anal canal are the anorectal junction and the anal margin. The puborectal band and the interspecific groove are identified upon palpation. The columnar or cylindrical epithelium of the rectum extends about 1 cm above the dentate line where the transition area begins. Below this line, the covering epithelium is exclusively scuamous. The anal margin is the pigmented tegument surrounding the anal opening, extending over a 5 cm area.

Specifying the point of origin is uncertain at the time of diagnosis, making it difficult to differentiate whether the origin is in the anal canal or anal margin. Thus, the origin of the tumor is at the intraanal, perianal or tegumentary level, outside the 5 cm area. Lymphoid nodules occupy an important role in diagnosis due to lymphatic dissemination (Sunesen et al., 2009).

The primary lymphoid station is at the level of perirectal nodules along the inferior mesenteric artery. The secondary station is at the internal pudendal nodes, above the dentate line and at the level of the internal iliac system. At the level of the inguinal, femoral and external iliac nodes, the lymph arrives from the lower portion of the dentate line and the skin of the perianal region (Sunesen et al., 2009).

For a complete assessment, staging was required, according to ESMO-ESSO-ESTRO 2014 (Sunesen et al., 2009), TNM that evaluate primary tumor (TX-primary tumor can not be assessed, T0-primary tumor absence, Tis-carcinoma in situ, T1-tumor ≤2cm size, T2-tumor> 2cm but ≤5 cm size, T3-tumor> 5cm, T4-any size and invade neighbor organs, eg vagina, urethra, urinary bladder), regional lymph nodes (NX - regional nodes can not be evaluated, N0 - no significant nodes, N1-metastasis at the level of perirectal nodes, N2-metastasis in unilateral iliac or inguinal nodes, N3-metastasis in perirectal and inguinal and / or bilateral nodes, internal iliac nodes and / or inguinal) and distant metastasis (M0-without metastases, M1-metastases at a distance).

Rectal cancer has multiple influences and presents a wide range of risk factors. Analyzing a group of people in the Asia / Pacific region, Morrison (Morrison et al., 2013) observed that in 158 cases of rectal cancer, advanced age and each year of life increased the risk by 8-9%. Parallel analysis revealed closerelations of CRC with BMI.

In a study by Promthet on a group of 112 patients diagnosed with rectal cancer and another group of 242 healthy people, it was noted that 22 patients experienced frequent constipation episodes versus only 4 in the control group, 43 patients showed hemorrhoids of varying degrees in their history, versus only 34 of the control group presented, 41 patients had a history of heredocolateral cancer, 47 of the patients frequently consumed pork meal (Promthet et al., 2012). Because of the percentage, these are comonly considered risk factors in rectal cancer.

In the study made by Iswarya in which she studied risk factors in colorectal cancer on two groups, control and study group respectively, it was noted that 22.3% of cases consumed eggs 2-3 times a week compared to 8.5% of control, consumption of sheep meat more than 2-3 times a week increased the risk seven times, and that eating fried foods more often 2-3 times a week increases the risk of rectal and colon cancer (Iswarya et al., 2016). In a review by Hjartaker on published studies on PubMed, it was noted that the frequent consumption of red meat, processed meat 22.3g or more per day, reduced dietary fiber consumption below 0.9g, are risk factors for cancer. The amount of Ca in the body, had an inverse proportionality relationship with rectal cancer (Hjartaker et al., 2013).

In terms of type of diet, Orlich observed the low incidence of rectal cancer in vegetarian diet followers: 40,367 vegetarians, 55 cases of rectal cancer. Of the vegetarian diet, vegetarian, pescovegetarian and semivegetarian lacto-ovo, pescogetarian has the lowest risk ratio, 0.57 compared to 1.37 for non-vegetarians (Orlich et al., 2015).

The study of Claessen in which 12 patients with rectal obstruction from subtotal colectomy were compared with 18 control patients showed that the influence of primary sclerosing colangitis and time period to subtotal colectomy on rectal cancer were considered risk factors (Claessen et al., 2009).

Anal cancer has a multitude of risk factors. Coffey analyzed a group of 1.3 million women and found 517 cases of anal cancer with a high incidence of squamous cell carcinoma (82%). Of these, 46% reported smoking, 59% used oral contraceptives, of which 33% used for a longer period of 5 years. CIN 3 in personal history is considered the highest risk factor, 1% present at recruitment. Smoking increases the risk of developing anal cancer, while more than 15 cigarettes / day twices the risk (Coffey et al., 2015).

Nulliparous women seem to have an increased risk of 60% compared to women with one or more births. Including ligation of the fallopian tubes was seen as a risk factor. Bertisch analyzes the risks presented in a group of 59 men diagnosed with anal cancer. Of these, 73% were homosexuals over the age of 35. The control lot consisted of 295 healthy men (Bertisch et al., 2013).

Smoking had an increased incidence in the patient group (69%) compared to the healthy group (48%), and represents a risk factor. The history of AIDS is more common in positive anal cancer cases (42%) than the control group (31%), but with a small statistical difference. The number of CD4 + cells decreased compared to the control group led to its determination as risk factor. Analyzing the relationship of HPV serological markers and the risk of anal cancer, they discovered the direct proportion of HPV16, HPV 18, 31, 33, 35, 45, 52, 58, 6, 11 anti-L1 antibody proteins.

In a study conducted in North America, Silverberg demonstratethe association of anal cancer with HIV on a group of 34,189 HIV + patients versus 11,4260 healthy patients. Among men, on 100,000 individuals, the incidence of anal cancer was 131 for HIV-infected homosexuals, 46 for other men, and 2 for HIV-negative males. In HIV-infected women, the incidence was 30/100000 compared to HIV negative women who did not show anal cancer (Silverberg et al., 2012).

Cheng analyzed the risk factors for male patients who have anal sex with other men and are infected with HIV. Of the 230 subjects, 56% had more than 10 partners and 22.6% more than 5 in the last 3 months. Following cytology, patients with atypical squamous cells of undetermined or high-grade significance had recurrent HPV infections and various subtypes (Cheng et al., 2018).

Malignant pathology, rectal and anal cancer have various presentation forms, the most common being hematochezia, chronic anemia, changes in defecation (constipation or diarrhea), abdominal or rectal pain, and sudden weight loss. Diagnosis of rectal cancer is based on rectal digital exam and rigid or flexible biopsy sigmoidoscopy for the histopathological examination of tumors located below 15 cm distal to the anal margin. Various screening methods have been proposed for the early discovery of rectal cancers.

The American Cancer Association proposes annual digital examination after the age of 40, the annual Hemocult test after the age of 50 and flexible sigmoidoscopy at 3-5 years for asymptomatic individuals presenting risk factors and, due to possible synchronous tumors, colonoscopy is recommended.

In the study by Ahmad on a group of 3915 patients, 2854 patients were investigated with rigid sigmoidoscopy and 2947 with flexible sigmoidoscopy (Ahmad et al., 2011). A higher rate of cancers detection and greater patient comfort was found with flexible sigmoidoscopy, suggesting the use of this type of sigmoidoscopy against the rigid one. CEA (carcinoembryonic antigen) and TIMP-1 (plasma tissue inhibitor of metalloproteinases-1) are correlated with the early detection of colorectal cancer, first proposed by Ahlquist in 2012 and reconfirmed by a study by Christensen in 2015 on a group of 32 patients, 24 with colon cancer and 8 with rectal cancer. This demonstrates direct correlation with CRC with 95% specificity. MRI (magnetic resonance imaging) and TRUS (transrectal ultrasonography) are used as diagnostic imaging modalities (Christensen et al., 2015).

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.

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. These can be achieved only if radiological team has competence in the implementation of virtual colonoscopy.

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 (Goh et al., 2007).

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 (Ghosn et al., 2015).

Magnetic resonance is a non-radiant imaging technique that is less rapid than CT but provides better soft tissue contrasts, shows the relationship of tumors to the rectum, identifies lymphatic and vascular nodes infiltration (Iswarya et al., 2016). The use of T2 mode allows a good visualization of the rectal wall structure: mucosal and submucosal hyperintense, muscularis propria hypointense, and adipose perirectal tissue hyperintense, mesorectal fascia as a linear, fine structure and hypointense.

An important role is given to the mesorectal fascia in tumor staging by assessing the extension to the structures of the rectum, in predicting the distance between the circumferential edge of the tumor and this fascia. It has been established that patients with a deeper invasion of one millimeter of the fascia have an increased incidence of recurrence, ~~a~~ shorter survival during resolution, and a lower life expectancy compared to those who did not show invasion of the fascia (Rao et al., 2007).

The post-operator has the ability to predict the therapeutic attitude according to the relationship with the mesorectal fascia, the sphincter complex, the vascular invasion, the depth of the invasion, and TRG (the degree of tumor regression) as suggested by MgGlone and Patel (MgGlone et al., 2014, Patel, 2012).

Transrectal ultrasound is an essential imaging exam for local assessment of rectal tumors. It can visualize tumor size, morphology, extension in the rectal muscle, location in relation to the anus and perirectal nodes. Rectal tumors exhibit a variety of ecogenicity, evolving with breakage of rectal wall layers. The invaded lymph nodes have a size greater than 5-7 mm, being hypoecogenic and round, and vascularization is chaotic and intense (Betge et al., 2012).

Current treatment focuses on complete tumor excision and prevention of local recurrence and metastases based on standardized surgery, chemotherapy and radiotherapy adapted to TNM grade. The goal is to increase survival and life expectancy while minimizing the risk of recurrence and metastasis simultaneously with avoidance of toxicity following radiotherapy and chemotherapy (Regnier et al., 2017).

Neoadjuvant therapy involves the combination of radiotherapy with chemotherapy and TME (total mesorectal excision), with beneficial effects observed by van Gijn on a group of 1,861 patients who have been monitored for 12 years, which has reduced the reemergence for 10 years due to neoadjuvant therapy consisting of radiotherapy, 45-50 Gy for 25-33 days, together with 5-fluorouracil-based chemotherapy, according to the ESMO Guidelines (Gijn et al., 2011, Glynne, 2017). Trials of oxaliplatin, irinotecan, leucovorin with better therapeutic effects, but with much higher toxicity have been attempted (Cortejoso and Lopez-Fernandez, 2012).

The moment of surgical intervention after neoadjuvant therapy is still uncertain. It is thought that late intervention during neoadjuvant therapy could result in a tumor that is too life-threatening, and would lead to tumor shrinking while complicating surgery due to fibrosis and postoperative morbidity. As considered by many authors, the optimal response time is 6-8 weeks due to the ease of shrinking and fitting at a lower TNM stage while reducing the risk of mortality caused by perianal complications (Sagar and Pemberton1996).

Postoperative treatment is no longer recommended, but there are cases when chemoradiological treatment is given: if not administated pre-operative, if there are positive circumferential margins, tumoral perforation or affected mozorectal structure. Breugom conducted a meta-analysis of the EORTC 22921, Chronicle and Italian I-CNRRT, PROCTOR / SCRIPT trials on a group of 1196 grade II and III patients and did not see benefits for 7 years, but in those with the tumor located 10-15 cm from the anal orifice, DFS (disease-free survival) increased, but without increasing life expectancy (Breugom et al., 2015).

Anal cancer or anal scuamocellular carcinoma is diagnosed histopathologically. 20% of patients have no symptoms, which requires a strict screening program. Common forms of presentation are bleeding, which requires differential diagnosis with hemorrhoids, mucosal ulcer that does not heal, mass, pain, pruritus, fistula or faecal incontinence (Ghosn et al., 2015).

Because of the viral determinat factor (HPV), vaccine prevention is being discussed (Stier et al., 2016). It is estimated that about 80% of cases can be avoided, but it does not provide protection for those already infected. According to ESMO (Glynne et al., 2017), clinical assessment involves rectal digital examination to assess the lesion and involvement of perirectal nodules. If the patient is a woman, due to infiltration of neighboring organs, vaginal exam is necessary to determine tumor size, vaginal septum involvement, persistence of a fistula, involvement of vaginal mucosa or possibile existence of formations.

Due to premalignant lesions (HSIL), high resolution anuscopy allows early diagnosis and early treatment. The technique involves the insertion of an anuscope and a colposcope that visualizes the scuamocellular junction, the anal canal and the perianal skin. Initial inspection will be done immediately after insertion, then use acetic acid 3% or 5% to highlight lesions. Examination is done under the 16X magnification then 25X when the lesions are visualized, and 10X at the anal edge. To differentiate HSIL from LSIL, Lugol solution is applied. HSIL appears as a flattened or thickened lesion with punctual vascular changes or as a mosaic, getting white to acetic acid with a slight absorption of Lugol solution. For certainty, biopsy is performed (Clavero, 2017).

In a study by Machalek of 400 biopsies from 283 patients over a 7-year period, there was an increase in the detection rate of HSIL, suggesting the efficacy of the method. In the case of the bioptic examination, the therapeutic path is surgical in the case of small sized lesions up to 1/3 of the circumference of the anal canal (Machalek et al., 2013). Ablation with infrared coagulation, electrocautery, cryotherapy, and photodynamic therapy are involved, but the Association of Coloproctology of Great Britain and Ireland, the American Society of Colon and Rectal Surgeons and the Italian Society of Colorectal Surgery do not agree on the treatment. Monitoring recommendations were make by Atkin, ranging from repeated anuscopy to 6 months to 1 year, with or without biopsy (Atkin et al., 2012).

Magnetic resonance imaging, as demonstrated by Jederán is used both in diagnosis and in patient monitoring, providing much more accurate data than endo-anal ultrasonography (Jederán et al., 2012). PET / CT (positron emission tomography) with fluorodeoxyglucose is highly sensitive to detect affected lymph nodes (Petersen et al., 2014).

The treatment aims is locoregional control and preservation of anal function. In the initial stage, chemoradiological treatment is recommended based on 5-fluorouracil and mitomycin C, considered standard treatment, which has as result tumor regression in 80-90% of cases. The option of surgical treatment as the initial step is plausible in lesions <2cm in diameter with safety margins <5mm without endangering sphincter function. Another therapeutic option is brachytherapy that consists of tumoral irradiation in the high dose of iridium-192, maintaining organ function, but associated with chemoradiotherapy. If after post-surgical assessments there is tumor tissue, chemoradiotherapy is administered (Tunio et al.,, 2010).

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, coloregional extension of the tumor and systemic dissemination of cancer cells should be known. Formulation of a therapeutic protocol and prognosis of colorectal cancer depends 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.

2.3.4.1. ***Angiographic study***

Comparison of microquantifying on histological sections, previously performed, with microvascular density on surgery specimen noted a significant correlation. Vascular index is considered a worse indicator for evaluating of lymphatic metastases and venous microinvasion. Although neoangiogenesis is an important step in tumor genesis, a prerequisite for tumor progression, is not the only factor that determines recurrence.

Evaluation of microvascular density may have errors due to heterogeneity of the micro vascular distribution. For this reason, it is necessary to analyze multiple images and sections of the 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 et al., 1998, Goh et al., 2007).

Clinical trials performed for tumoral staging correlates high intensity of tumor genesis with tumor aggressiveness, high microvascular density having a predictive role in development of metastases. *On the same material, we performed studies regarding tumoral microvessels, by immunohistochemistry and quantitative microanatomical 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.

2.3.4.2.*Imunohystochemical, MRI and CT studies*

The muscular layer has irregular grooves steering, but uniform in size due to 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.

Finally, we observe another zone with reduced MRI signal, which surrounds the rectum and the perirectal fat. This is fascia mesorectum. Any changes of these anatomic and radiological issues should be suspected and investigated for presenting paraneoplastic origins.

The blood and lymphatic vessels have a rich density to their home area (posterior and mesorectal), which decrease as we head towards anterior. This distribution is consistent with vascular predisposition of forming a rectal cancer, especially on the rear wall or possibly on the side.

The establishment of a normal vascular pattern of rectum was obtained 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 resection pieces.

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

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 do 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.

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).

2.3.5. Conclusions

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.

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 of clear changes in caliber and trajectory of rectal vessels.

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.

2.4. FIELDS FOR FUTURE RESEARCH ACTIVITY

2.4.1. FUTURE RESEARCH PROJECTS

2.4.1.1. *Interrelation between parathyroid and thymus glands in the Miastenia Gravis*

State of the art

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 (Gilmour 1937, Mahadeva 2008, Liu 2010, Nicolle 2016,).

On the other hand, Gcm2, specifically required only in parathyroid organogenesis, but still present after parathyroidectomies led to the assumption that thymus could act as a secondary source of PTH. It was proven to be inaccurate.

A later research explained this phenomenon by proving that the thymus parathyroid separation is "messy" with clusters of parathyroid cells remaining throughout the neck region and transdifferentiating in small *cervical* thymi which produce the PTH (Okabe 2004, Neves 2012, Gordon 2011, Gardiner 2012).

Research shows that thymic epithelial cells (presenting only AChR subunits) *"prime"* T-helper cells, thereby prompting antibody development against thymic myoid cells (express fully formed, clustered AChR). These in turn will diversify to recognise intact muscle AChR (Gordon 2004, Gordon 2010, Dooley 2006, Terszowski 2006).

Klinger (Klinger et al. 2012, Liu 2006, Liu 2005) have found that the parathyroid hormone prompts human T cell activation:

-PTH acts on T cells

-Acute exposure to PTH augments PHA-induced T cell proliferation and IL-2 production

-This action of PTH is related to its biological activity and is most likely due to the ability of PTH to enhance entry of calcium into cells.

We thus believe that this is a correlation, as T cells are activated by PTH, which in turn activate AchR AB formation.

It is characterized by its fluctuating degree and variable combination of weakness and fatigability in ocularptosis, bulbar, limb and respiratory muscles. This weakness is a result of an antibody mediated attack directed at proteins in the postsynaptic membrane of the neuromuscular junction E.g. the acetylcholine receptor proteins(AChR).

Role of Thymus in Myasthenia Gravis:

- these auto-antibodies are thought to originate in the hyperplastic germinal centers of the thymus where myoid cells expressing AChR are clustered.
- they are the only known cells to express intact AChR outside of muscle.
- thymic epithelial cells produce AChR subunits that are hypothesized to prime helper of T cells.
- the T cells then attack the AChR on myoid cells in the hyperplastic thymus.

- the autoimmunization completes as the antibodies diversify to recognize intact muscle AChR .
- the majority of patients with AChR antibody positive myasthenia gravis have thymic abnormalities:
  - hyperplasia in 60 - 70 %
  - thymoma in 10 - 12 %.

· The disease's clinical signs often improve or disappear after thymectomy.

Embryological development of Thymus and Parathyroid

Both arise from the endoderm of the 3rd and 4th pharyngeal pouches. *Initial parathyroid organogenesis is closely linked to thymus organogenesis.* These organs arise from different regions of the same pouches, and during development they undergo a series of morphogenetic events to form separate organs. The thymus and parathyroid domains separate from each other by a less well understood mechanisms, but involvement of neural crest cells prompts thymus migration, dragging along the inferior parathyroid glands (Dubner, 2017).

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.

Physio-pathological correlation

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.

On the other hand, Gcm2, specifically required only in parathyroid organogenesis, but still present after parathyroidectomies led to the assumption that thymus could act as a secondary source of PTH. It was proven to be inaccurate.

Meaningful correlation between these are about parathyroid adenoma/thymoma related cases, myasthenia gravis associated with primary hyperparathyroidism, parathyroid hyperplasia associated with thymoma, parathyroid adenoma associated with thymoma in a female adult with primary hyperparathyroidism, primary hyperthyroidism due to a parathyroid adenoma with subsequent myasthenia gravis and resolutions of primary hyperparathyroidism following surgical removal of cervical thymus.

Iași thoracic surgery department database shows that 57.14% of patients who had myasthenia gravis presented a form of thymic tumour (thymoma, hyperplasia, thymic nodule and carcinoma) and 7.14% presented hypothyroidism. No PTH were registered in the database before or after thymectomy.

The purpose of this study is to determine and quantify the existence of a functional bond between the anterior parathyroid glands and the thymus. We intend at the same time to study this relationship in the pathological context of the Myasthenia Gravis, in collaboration with the Thoracic Surgery Clinic in Iasi.

2.4.1.2. *Artistic hearing*

State of the art

Along with the development of human musical culture over time, the term of “musical ear” has emerged. It refers to the fact that some individuals perceive the sound waves differently than the great mijority. They retain and reproduce more easily a sound they first hear.

The notions of neurolaryology and neuroaudiology are closely correlated with those of phoniatry.

Current studies show differences in the perception of sound waves between the left and right ears. The auditory cortex exhibits different changes when a subject listens to melodic sounds of different intensities (Fiser, 2010, Lima, 2019).

Meanwhile, other studies show that there are changes in the sound perception ability of people who have musical training. This refers to the fact that those who play musical instruments, vocal singers, conductors etc. have a much higher discriminative perception of sound than a person who listens to an occasional song (Parrot, 2014, Liang, 2016).

This project will be run in parallel with the study of vocal formants. The main method of work will be 3D-CBCT through which we can acquire linear and volumetric data about the middle and internal ear in singing artists.

We will use a control lot of subjects without specific vocal training.

All subjects will be required to follow the same protocol as in the study of the superior vocal formant. Thus, we will be able to detect the changes occurring within the middle and internal ear in these subjects compared to the control group.

By applying 3D reconstruction and printing techniques, we will be able to identify the morphological and, implicitly functional acquired or inborn features of them.

2.4.2. IMPLEMENTATION PLAN

I am looking forward to begin both projects before closing the ones already in progress, ie within two years. To carry out these projects, I intend to apply for funding by submitting national and international grant projects.

2.4.3. THE ANTICIPATED IMPACT OF IMPLEMENTING NEW RESEARCH DIRECTIONS ON THE ACADEMIC CAREER AND PERSONAL INTERNATIONAL VISIBILITY

Possible approval of the funding of the proposed objectives will also allow me to disseminate the results of my research.

Achieving these goals allows the improvement of vocal training techniques by focusing on structures with the most active role in determining vocal abilities and faster and accurate accomplishment of appropriate motor patterns by exploiting vocal techniques to direct stimulation of hot zones.

The results of this study are directly applicable to the teaching staff involved in teaching of vocal techniques and students who wish to achieve a career in the field of both theater and singing.

The results of the study on clinically healthy subjects has a direct applicability in the field of otorhinolaryngology, and especially in the field of phoniatry and audiology, through the targeted orientation of prophylaxis techniques of diseases of the phonoauditory apparatus on the anatomical structures with high risk.

Disseminating results by publishing articles in ISI journals on yellow or red areas will increase my international visibility as a researcher in the field.

At the same time, I propose to conclude a long-term collaboration with ”George Enescu” University of Arts in Iaşi, with the idea ofimplementing phoniatrics and functional anatomy classes of the phonatory apparatus in the curriculum for students from the singing and theatrical sections.

In the same context, I would like to propose a postgraduate course of phoniatrics and audiology with an artistic involvement, to be developed with my colleagues from otorhinolaryngology.

2.4.4. FURTHER RESEARCH OF PROJECTS ALREADY STARTED

2.4.4.1. *Directions and principles for further research of SMAS*

The main direction of research that continues and uses the results of the SMAS study is Moebius Syndrome.

The project that I propose and where I have already started to work on is called *Aging Moebius* and combines the concepts of SMAS with that of congenital atresia of facial nerve.

Practically, the project aims to re-evaluate patients with Moebius syndrome and assess any new cases. These patients will be classified according to the type of facial nerve damage, which is a criterion for establishing a clinical and paraclinical assessment protocol.

At the same time, I propose to associate this protocol with specific genetic tests to detect a possible genetic sequence involved in the morphopathology of this disease.

2.4.4.2. *Incentives, directions and principles to continue research on phonatory apparatus*.

A. *The main area of interest in this regard and the next step is to carry out a follow-up project of the initial research using the Functional MRI technique. This will be feasible only after the detection of those HOT-SPOTs from the mouth and palatine veil that are the triggering sites ofthe neural reflex of returned impedance of the larynx by the pavilion.*

The effects of changing stimulus durations could be examined for speech and nonspeech signals. The aperiodic initial portion of the syllable [•n] is cut by progressive 7-msec decrements thus generating stimuli perceived as progressing, as expected, through several categories lrom unvoiced sibilant to voiced stop.

Recent findings that the speech dominant hemisphere is specialized at the level of distinctive feature analysis entail a prediction that the processing of acoustic cues embodying a feature distinction in a particular language is asymmetric for "native" listeners, but not asymmetric for nonspeakers of the language if their own language does not employ the contrast.

Electrophysiological validation of fMRI connectivity analyses is realised based on Granger causality and Dynamic Causal Modelling using a well-characterised animal model of functional coupling. It has important implications for neuroimaging map of acoustic reflexes. It could indicate that one must minimise spurious interactions due to hemodynamic variability between brain regions using explicit or implicit (such as in DCM) deconvolution of hemodynamic effects in fMRI time series.

B. *The second step consists of conducting experiments on mucosa and vocal muscles in order to detect types and subtypes of nerve receptors at that level. I would try to realise these studies through Electron Microscopy techniques correlated with Functional MRI.*

The theory of propagation of weakly nonlinear waves through random media is discussed. The wave field is partitioned into mean (ensemble average) and fluctuating components, and a nonlinear equation is derived governing the evolution of the mean field. This equation contains a pseudo viscous term that takes account of the irreversible transfer of energy from the mean wave to the fluctuating field.

By balancing nonlinear effects against this dissipation, the existence of steady-state shock like waves is deduced for the mean field, and this is illustrated by reference to the propagation of sound through an atmosphere in which the sound speed is a random function of position and through one subject to turbulent fluctuations.

An implicit finite difference method has been developed for calculating spherically symmetric nonlinear acoustic flows generated by a high intensity periodic source. This method makes it possible to match the conditions prescribed at the source to a low amplitude solution (in Blackstock's sense) valid at large distances from the source. Numerical computations have been made for pistonlike and sirenlike sources of three different periods.

The larynx is a highly reflexogenic area, and stimulation with mechanical and chemical stimuli results in a number of protective reflexes. Investigators have used anatomical, behavioral, and neurophysiological techniques to examine the receptors responsible for initiating these reflex responses.

Histologic examination has revealed the presence of free nerve ending, Merkel cells, Meissner corpuscles, and taste buds. Mechanoreceptors have been classified in several different ways and are located either in the superficial mucosa or in muscles and laryngeal joints.

Recordings from afferent fibers innervating laryngeal mechanoreceptors have revealed that some of them are spontaneously active whereas others are silent until stimulated.

Laryngeal mechanoreceptors respond to stimulation with either a rapidly adapting or a slowly adapting response pattern. Often the mechanoreceptors respond to respiratory movement of the larynx, giving bursts of action potentials during inspiration. A large number of taste buds that are anatomically similar to lingual taste buds populates the laryngeal surface of the epiglottis. Taste buds of the larynx respond to a number of chemical stimuli and to water.

2.4.4.3. ***Directions and principles for further research of neovasculogenesis processes in colorectal cancers***

I would like to continue research in this field by studying the phenomenon of paraneoplastic neovasculogenesis in parallel, this occuring in the most frequent types of cancers located in the inframezocolic floor of the abdomen and at the perineal level.

Minimally invasive surgery is becoming increasing popular in the treatment of CRC. Nam et al. looked at using a minimally invasive approach for both colon and rectal cancers. Typically, a minimally invasive approach allows for faster recovery of bowel function, reduced post op pain and shorter hospitalizations. Laparoscopic colon resections are widely done for colon cancer, however, for rectal cancer the operation is technically more difficult due to dissection in the deep and narrow pelvis. Robotic surgery is becoming more popular for rectal cancer as it allows for better visualization in the pelvis and improved ergonomics and dexterity when compared to laparoscopic surgery for total mesorectal excision.

Chemotherapy has evolved in the past 20 years and now there are multiple combination regimens available. The most popular include FOLFOX (leucovorin, 5-FU, and oxaliplatin), FOLFIRI (leucovorin, 5-FU, and irinotecan), and in some cases a drug that targets VEGF or EGFR, bevacizumab (Avastin) or cetuximab (Erbitux) respectively. Different combinations of chemotherapy and radiation are given to patients based on stage, tumor characteristics and presence of biomarkers.

Due to advances in the treatment of CRC and early detection, the survival rates have increased with a 5-year survival of 65% at all stages.

To this regard, I consider the study of these aspects in the case of metastasis of pancreatic cancers, primitive retroperitoneal tumours and females genital cancers by angioMRI, angioCT and PET-CT techniques.

Efforts should be made for increasing formal screening programs in appropriate population. Identifying people at higher risk because of family history such as inflammatory bowel disease, or genetic conditions and targeting them for screening should be encouraged. Finally, educating individuals on risk factors and what should prompt them to seek care combined with a multi-disciplinary approach will help tackle the rising CRC burden and improve outcomes.

CHAPTER 3

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