GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

HABILITATION THESIS

INTERDISCIPLINARY RESEARCHES ON TUMORAL AND INFLAMMATORY DISEASES OF THE EYE AND OCULAR ADNEXA

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CONTENTS

ABBREVIATIONS

ACTH - adrenocorticotropic hormone
AMs - atypical meningiomas
AMT - amniotic membrane transplant
AD - acute dacryocystitis
BCC - basal cell carcinoma
BRST-1 - monoclonal mouse anti-human BCA225
Cam 5.2. - cytokeratin marker
CD - chronic dacryocystitis
CEA - carcinoembryonic antigen
CIS - chronic inflammatory score
CK - cytokeratin
CNS - central nervous system
CoNS - coagulase-negative staphylococci
CT - computed tomography
DCR - dacryocystorhinostomy
EGF - epidermal growth factor
EMA - epithelial membrane antigen
F - female
FCAT - Federative Committee on Anatomical Terminology
FD - fibrous dysplasia
HA - hydroxyapatite
HE - Hematoxylin–Eosin
HLA - human leukocyte antigen
ICP - intracranial pressure
IFN- γ - interferon- gamma
IJCN - inflammatory juvenile conjunctival nevus
IH - intracranial hypertension
IHC - immunohistochemical
LI - labelling index
LDS -lacrimal duct system
LS - lacrimal sac
LSCD - limbal stem cell deficiency
LSCT - limbal stem cell transplantation
M - male
MALT - mucosa- associated lymphoid tissue
MMC - micrographic surgery
MPh - microporous polysacharide hemispherre

MRI - magnetic resonance imaging
NC - nasopharyngeal carcinoma
NLD - nasolacrimal duct
NLDO - nasolacrimal duct obstruction
NGF - nerve growth factor
ONS - optic nerves
PFS - progression – free survival
pBCC - periocular basal cell carcinoma
PE - polyethylene
PFS - progression free survival
PMMA - polymethyl- methacrylate
PTFE - polytetrafluoroethylene
RNFL - retinal nerve fiber layer
SGC - sebaceous gland carcinoma
SPSS - statistical package for the social sciences
TA - Terminalogia Anatomica
TDM - tomodensitometry
TNF - α - tumor necrosis factor- alpha
UV - ultraviolet
WHO - World Health Organization

ABSTRACT

My academic, scientific and professional activity during the 15 years after obtaining the title of Doctor of Medical Sciences, Ophthalmology specialty is briefly reported in the thesis work. It contains three sections divided into chapters and subchapters that highlight the results of my academic and medical career.

Section I covers my scientific, academic and professional work.

Chapter 1 presents a summary of my scientific, academic, and professional achievements.

Chapter 2 contains subchapters in which my work on the treatment of tumors and inflammatory diseases of eye, ocular adnexes and orbit are presented. The tumoral diseases diagnosed more and more frequently in adults and children is an important responsibility in carrying out medical assistance, because the implications of the treatment are often surgical, leading to excision of the tumor together with part of the ocular adnexes affecting thus the aesthetic aspect and mental state of the patient. Among the most common tumor pathologies studied, treated and presented in this chapter of the habilitation thesis are: malignant and benign eye, periocular and orbit tumors. I presented research on periocular carcinomas, the correlation between the clinical manifestation and histological investigations on different types and subtypes of carcinomas. Regarding this pathology, I also presented a clinical study on periocular basal cell carcinomas and its subtypes. I also performed studies on the anatomical and anatomo-clinical aspects of sebaceous glands carcinoma as well as its surgical treatment. I also mentioned in this section the anatomical-clinical aspects of the cutaneous horn of the eyelids and of the compound juvenile nevi of the eye conjunctiva. Research on the oncological diseases of malignant melanoma and choroidal metastases was completed with scientifically important conclusions on the clinical, surgical and immunohistochemical aspects of these oncological disorders of the eye. Regarding the diseases of the orbit, I investigated the venous cavernous malformations both from the point of view of their clinical and surgical aspects. The pathology of rhino-sinus tumors with extension in orbit has been the subject of my concerns, because secondary exophthalmos is often found as an ophthalmologic sign of the disease.

In chapter 3, I presented the results of research on inflammatory ophthalmic diseases, such as chronic dacryocystitis, and a study on the methylene blue highlighting of the lacrimal sac in dacryocystectomy.

Chapter 4 deals with ocular prostheses and the reconstruction of the cornea and conjunctiva by applying the amniotic membrane transplant, in diseases of the ocular surface, which I applied with remarkable results. I conducted researches on the chemical and immune-histochemical properties as well as ultrastructural changes of the human amniotic membrane under the action of UV rays and antibiotic treatment.

Chapter 5 contains the results of researches on the neuro - ophthalmic anatomical structures, optic chiasm and the sphenoid bone.

Chapter 6 includes researches on neuro-ophthalmic diseases. Thus, following the accident at the Chernobyl nuclear power plant in 1986 which was manifested by a radioactive cloud, which affected quite strongly the NE area of Romania, where the region of Moldova is located, contributed to the increase of the incidence of meningiomas. Orbito-frontal fibrous dysplasia with visual loss was another aspect researched and presented in this chapter.

Chapter 7 includes clinical studies on neuro-anatomical terminology as well as research on the icono-diagnosis of surgical diseases. The results of the research presented in Section I of the Habilitation Thesis were published in ISI journals with impact factor and in BDI journals. The recognition of my research is confirmed by the number of citations of over 500 published articles as well as an H index = 12.

Section II, Future Career, Scientific and Academic Career Development Plans is structured in four subchapters that highlight five directions of development. 1. The development of my professional career is essential to me and I put constant efforts for improvement, in order to heal patients in the shortest possible time. 2. I structured the development of my research career along five directions: ophthalmic oncology, reconstruction of the ocular surface and prosthesis of the orbital cavity, interdisciplinary research with ophthalmological implications, neuro-ophthalmology, icono-diagnosis and neuro-anatomical terminology. The purpose of my present and future researches is to improve my medical and scientific activity. 3. The development of my academic career aims at improving the theoretical and practical teaching and learning techniques for students and resident doctors within the Ophthalmology Discipline of the “Grigore T.Popa” University of Medicine and Pharmacy of Iași, Romania. This section also includes conclusions regarding my academic, research and professional activity exposed in the Habilitation Thesis.

Section III contains bibliographic references.

REZUMAT

Activitatea mea academică, științifică și profesională desfășurată în decursul celor 15 ani după obținerea titlului de Doctor în Științe Medicale, specialitatea Oftalmologie este relatată pe scurt în lucrarea teza de abilitare. Aceasta conține trei secțiuni împărțite pe capitole și subcapitole ce reliefează rezultatele preocupărilor mele în cursul carierei universitare și medicale.

Secțiunea I cuprinde activitatea mea științșifică, academică și profesională. Capitolul 1 prezintă o sinteză a activității mele de cercetare științifică, academică și profesională. Capitolul 2 cuprinde subcapitole în care sunt prezentate preocupările mele cu privire la tratarea bolilor tumorale ale ochiului și anexelor sale dar și ale orbitei. Diagnosticarea și tratamentul bolilor tumorale și inflamatorii diagnosticate din ce în ce mai frecvent la adulți cât și la copii este o responsabilitate importantă în desfășurarea actului medical, deoarece implicațiile tratamentului de cele mai multe ori chirurgical, conduce la excizarea tumorilor împreună cu o parte din anexele globului ocular afectând astfel, aspectul estetic și starea psihică a pacientului. Printre cele mai frecvente patologii tumorale studiate, tratate și prezentate în cuprinsul acestui capitol din Teza de abilitare sunt: tumori maligne și benigne oculare, perioculare și orbitale. Am prezentat cercetările cu privire la carcinoamele perioculare, corelația dintre manifestarea clinică și investigațiile histologice privind diferite tipuri și subtipuri de carcinoame. Cu privire la această patologie am analizat și un studiu clinic cu privire la carcinomul bazocelular al pleoapelor și subtipurile lui. De asemenea, am efectuat studii cu privire la aspectele anatomice și anatomo-clinice ale carcinomului de glande sebacee, precum și tratamentul lui chirurgical. Am menționat in această secțiune și aspectele anatomo-clinice ale cornului cutanat al pleoapelor și ale nevilor juvenili compuși conjunctivali. Cercetările cu privire la melanomul malign ocular și la metastaza coroidiană au fost finalizate cu concluzii importante din punct de vedere științific privind aspectele clinice, chirurgicale și imunohistopatologice ale acestor boli oncologice ale ochiului. In ceea ce privește patologia orbitei, am cercetat malformațiile cavernoase venoase, atât din punct de vedere al aspectelor clinice, cât și chirurgicale ale acestora. Patologia tumorilor rino-sinusale cu extinderea orbitală a făcut obiectul preocupărilor mele, deoarece exoftalmia secundară este constatată de cele mai multe ori ca semn oftalmologic al bolii. In capitolul 3 am prezentat rezultatele cercetărilor mele cu privire la bolile oftalmologice inflamatorii, cum este dacriocistita cronică, și un studiu cu privire la evidențierea cu albastru de metilen al sacului

lacrimal in dacriocistectomie. Capitolul 4 se referă la proteze oculare și reconstrucția corneei și conjunctivei prin utilizarea transplantului de membrană amniotică, în bolile suprafeței oculare, pe care am aplicat-o cu rezultate remarcabile. Am efectuat cercetări cu privire la proprietățile chimice și imunohistochimice, precum și modificările ultrastructurale ale membranei amniotice umane, sub acțiunea razelor UV și a tratamentului cu antibiotic. Capitolul 5 cuprinde rezultatele cercetărilor mele cu privire la anatomia structurilor neuro-oftalmologice, chiasma optică și osul sfenoid. Capitolul 6 cuprinde cercetări cu privire la bolile neuro-oftalmologice. Ca urmare accidentului produs la centrala atomo-electrică de la Cernobâl in anul 1986 și care s-a manifestat printr-un nor radioactiv, ce a afectat destul de puternic zona de NE a României, în care este situată regiunea Moldova, a contribuit la creșterea incidenței meningioamelor intracraniene. Displazia fibroasă orbito-frontală, cu afectarea acuității vizuale a fost un alt aspect cercetat și prezentat la acest capitol. Capitolul 7 cuprinde studii clinice cu privire la terminologia neuroanatomică precum și cercetări cu privire la icono-diagnosticul unor boli chirurgicale. Rezultatele cercetărilor prezentate in Secțiunea I a Tezei de abilitare au fost publicate in reviste ISI cu factor de impact, precum și în reviste BDI. Recunoașterea cercetărilor mele sunt confirmate de numărul de citări ale articolelor publicate de peste 500 precum și un H index = 12.

Secțiunea II, planuri viitoare de dezvoltarea carierei profesionale, științifice și academice este structurată pe patru subcapitole care reliefează cinci direcții de dezvoltare. 1.Dezvoltarea carierei mele profesionale este o componentă importantă a carierei mele, pentru care am preocupări permanente de perfecționare, cu scopul de a vindeca pacienții in timpul cel mai scurt posibil. 2.Dezvoltarea carierei științifice am structurat-o pe domeniile care au constituit preocupările mele de până acum pe cinci direcții de cercetare: oncologia oftalmologică, reconstrucția suprafeței oculare și protezarea cavității orbitare, cercetări interdisciplinare cu implicații oftalmologice, neuro-oftalmologia, icono-diagnostic și terminologia neuro-anatomică. Scopul cercetărilor mele prezente și viitoare este de a perfecționa pregătirea mea din punct de vedere medical și științific. 3. Dezvoltarea carierei mele academice vizează perfecționarea tehnicilor de predare teoretice și practice și de învățare pentru studenții și medicii rezidenți din cadrul Disciplinei de Oftalmologie a Universității de Medicină și Farmacie “Grigore T.Popa” Iași, România.Această secțiune cuprinde și concluzii cu privire la activitatea mea academică, științifică și profesională expusă în teza de abilitare.

Secțiunea III cuprinde referințele bibliografice.

SECTION I - ACHIEVEMENTS IN SCIENTIFIC, PROFESSIONAL AND ACADEMIC ACTIVITY

I.1. Introduction

The habilitation thesis highlights my post-doctoral scientific, personal and academic activity over a period of 15 years.

Academic activity - I started my academic career in 2005 when I obtained via competitive examination the position of junior assistant professor in Ophthalmology at the "Grigore T.Popa" University of Medicine and Pharmacy of Iași, Romania. My academic career built up through promotions, occupying through competitive examinations the positions of assistant professor (2008), lecturer (2018) and associate professor (2019). I started my doctoral studies in 2004 after having passed successfully the entrance exam. The supervisor for writing the doctoral thesis with the topic: "Considerations on various methods of treatment of eyelids tumors", was Prof. PhD MD Sergiu Buiuc. After the public defense of the doctoral thesis, I obtained the Doctorate Diploma in Medical Sciences, Ophthalmology specialty, with No. 1404 / 17.04.2008, issued by Order of the Ministry of Education, Research and Youth No. 3439 / 12.03.2008. I participated as a co-author in the elaboration of nine books and book chapters, the most significant being: 1.*Guide to Ophthalmic Pathology*. *Eyelid and conjunctiva diseases*, Anca Sava, Claudia Florida Costea, Florence Gabriela Dumitrescu, Ed. Î.E.P. Science (Publishing House of the Academy of Sciences of the Republic of Moldova), Chișinau, Republic of Moldova, 2015, ISBN 978-9975-85-007-0, 428 pages. 2.*Guide de pathologie ophthalmologique*. *Pathologie des paupières et de la conjunctive*, Anca Sava, Claudia Florida Costea, Florența Gabriela Dumitrescu, Universa Publishing House 9230 Wetteren, Belgium, ISBN 978-90-6281-028-4, D / 2015/0051/003, 442 pag. In order to improve the teaching system of the third-year students in Dentistry Faculty of "Grigore T.Popa" University of Medicine and Pharmacy of Iași, Romania. I developed power-point presentations in the following languages: Romanian, French and English, which I updated with novelties in the field of Ophthalmology. I am currently teaching courses in the Ophthalmology Discipline for third year students, program in Romanian, English and French. I also teach courses to resident ophthalmologists and to those from other disciplines. I am currently the Coordinator of the resident doctors from the Second Ophthalmology Clinic of the "Prof.Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania. I have been an elected member of the board of Department II Surgery of the "Grigore T. Popa" University of Medicine and Pharmacy of Iași, from 2016 to date. I

participated as a member in doctoral admission commissions, commissions for filling the positions of specialist and senior doctors of ophthalmology, professional promotions in the Ophthalmology Discipline of the "Grigore T. Popa" University of Medicine and Pharmacy of Iași. I taught within the Ophthalmology Discipline for 17 years. I participated in the elaboration of textbooks in Ophthalmology. I participated as a member in undergraduate commissions at the "Grigore T. Popa" University of Medicine and Pharmacy of Iași. I coordinated the activity of the resident doctors in Ophthalmology discipline assigned to the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, and in the specialized competitive examination and the senior consultant's competitive examination in the Ophthalmology Discipline at the "Grigore T.Popa" University of Medicine and Pharmacy of Iași, Romania. I supervised the academic writing of bachelor's theses by undergraduates of the Faculty of Medicine.

Professional activity **-** I started my professional activity in 2005 as a resident doctor and then in 2010 as an ophthalmologist in the Second Ophthalmology Clinic of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania, having passed successfully the admission competitive examination, based on Order no. 416/2010, no. 4991 / 03.05.2010 issued by the Minister of Health. Since 2018, I am a senior ophthalmologist at the Second Ophthalmology Clinic of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, as I passed the senior consultant's competitive examination and obtained the Certificate no. 15903 of 07.08.2018, Series PI no.018429, issued by Order of the Minister of Health, No.897 of 07.08.2018. I carried out my professional activity on multiple levels to acquire a solid professional training and solid surgical skills in anterior segment surgery, strabismus and oculoplasty. I underwent training courses in the country and abroad as follows: postgraduate training courses by participating in 14 international congresses and two national congresses held in London, Berlin, Barcelona Lisbon, Copenhagen, Paris, Geneva, Bucharest and Iași. I attended WetLab training courses in Austria - in Vienna and Schruns under the supervision of Sir John Bolger, PhD MD, a member of the Royal College of Surgeons of Ophthalmologists in London. At "Uma Eye Clinic" in Chennai, India, I completed 4 internships in cataract surgery by phacoemulsification technique and corneal surgery by LASIK technique where I performed 220 surgeries as a volunteer. In order to solve complex cases, we carried out interdisciplinary collaborations with pathologists, neurosurgeons, neurologists, plastic surgeons, gynecologists, radiologists, pediatricians and internists from teaching hospitals in Iași, Romania. I graduated the course Health Services Management, 29.10.2015-20.02.2016,

obtaining the Graduation Certificate no. 184 / 14.04.2016, AT Series, Nr. 0209, issued by the National School of Public Health, Management and Improvement in Health Bucharest, Romania. From a professional point of view, as a senior ophthalmologist, I am required to participate in surgeries of highly complex diseases (orbital tumors in children and adults) that required interdisciplinary teams consisting of primary neurosurgeons, plastic surgeons, maxillo-facial surgeons in the Hospital Emergency Clinic Prof. Dr. Nicolae Oblu, Iași and St. Mary's Children's Hospital in Iași, where I work as a volunteer whenever I am asked. I am also a member of the ethics committee of the "Prof.Dr.N.Oblu" Emergency Clinical Hospital Iași. I also participated in the organizing committees of national congresses and conferences:

Research directions **-** My scientific research activity carried out during the 18 years results in the publication of 39 scientific articles as main author, as well as 18 scientific articles as co-author published in ISI-rated journals with high impact factor: Lancet Oncology (IF = 35.386), International Journal of Molecular Sciences (IF = 4,183); CNS & Neurological Disorders - Drug Targets (IF = 2.76), Neurosurgical Review (IF = 3.042), Materials (IF = 3.057), World Neurosurgery (IF = 1.723), Diagnostic (IF = 3.706, Journal of Diabetics Research, Hindawi) IF = 2.965), International Journal of Molecular Sciences (IF = 4.556), Nutrients (IF = 4,171), RJME (IF = 1,033). I have published 64 main author articles and 16 co-author articles in BDI indexed journals. Thus, we achieved an FCIP = 51,717 and Hirsh index = 12. Papers published at congresses and other scientific events - 128. I am a reviewer in 13 international journals for which I conducted a number of 102 peer-reviews. My research interests and the elaboration of specialized books in ophthalmology resulted in a rich publishing activity also because I managed to involve Ophthalmologist doctors and professors from the Ophthalmology Clinic of the "Sf. Spiridon" County Emergency Clinical Hospital Iași, Romania, Second Ophthalmology Clinic of the Emergency Clinical Hospital Prof.Dr. Nicolae Oblu, Iași, România, as well as colleagues from other specialties such as: neurosurgery, anatomo-pathology, neurology, endocrinology, ENT, radiology, etc. I collaborate with ophthalmologists from the academic centers of Bucharest and Oradea. I obtained the Patent no. 130824 B1, no. application: 2015 00393, filing date 11/06/2015, date of publication of the patent: 29/05/2020, BOPI no. 5/2020, State Office for Inventions and Trademarks, Bucharest, 29.05.2020, Product for treating skin lesions and rheumatic diseases of the joints in collaboration with Țimpău Petru Mircea and Țimpău Ana Monica. The recognition of my scientific research activity has been rewarded by prestigious organizations on the occasion of participating in international and national congresses.

Chihaia M.A., Dimitriu G., Dumitrescu A.M., Dumitrescu G.F., Sava A., Costea C.F., Methylenblauverfärbung des Tränensacks bei Dakryo-Zystektomie - Falldarstellung, Konferenz Deutsche Ophthalmologische Gesellschaft Germania 2016, Abstract A-78-00. Paper awarded by the Board of Congress DOG Berlin 2016, for the best abstract admitted among the participants of Eastern European countries (DOG Travel Awards).

Claudia Florida Costea, Reviewer Honorary Certificate, for voluntary contribution and participation in the peer review for International Journal of Ophthalmology (IJO) from Nov.2017 to Oct.2018. President and Chief Editor, International Journal of Ophthalmology Press, Prof. Xiu-Wen Hu, President and Chief Editor, December 1, 2018.

Claudia Florida Costea, Anca Sava, Mihaela Mitrea, Roxana Popescu, Mihaela Dana Turliuc, Certificate of appreciation for the oral presentation "Eye socket - anatomical landmarks from a surgical point of view" at the 23rd Congress of the Balkan Dental Society (BaSS), Dental medicine of the younger vs. elderly patients, President of the Congress - Prof. PhD DMD Norina Consuela Forna, Prof. PhD DMD Argirios Pissiotis, Iași, Romania, 10-12 May 2018.

The article: De Santo NG, Bisaccia C, De Santo L, Cucu AI, Costea CF. John XXI, the Pope philosopher and physician-scientist of portuguese origins died of crush syndrome in 1277. Journal of Religion & Health, 2021, 60(2): 1305-1317, was rated 1*.1* the best evaluation, 25.09.2020.

Academic and Research Career Development Directions

The main directions of development of my career are: 1.Tumor and inflammatory diseases: eye and ocular adnexal tumors; neuro-ophthalmology; inflammatory diseases; 2.Eye reconstruction, use of amniotic membrane transplantation for reconstruction of the ocular surface. Eye prostheses; 3.Ophthalmology and neurosurgical and oral-maxillofacial interdisciplinarity; 4.Neuro-ophthalmology; 5.Icono-diagnosis and neuro-anatomical terminology.

These directions for the development of my career are the permanent guide for carrying out activities in the fields: ophthalmological research and teaching activity with students resident and doctors. The development of the scientific research activity focused on the five directions is structured as follows:

- Ophthalmic and interdisciplinary scientific research through collaborations as related medical specialties.

- Capitalizing on the outcome of our research in workshops, postgraduate courses and scientific events. Publication of results in ISI and BDI indexed journals and scientific papers at national and international conferences. To date, we have published articles with research results in prestigious international journals. Recording over 500 citations of the published articles, we noticed with satisfaction an interest in the outcome of our research from prestigious universities in the world.

-Promotion of scientific papers at national and international conferences, congresses.

-Coordination of bachelor's theses in Ophthalmology.

-Orientation and guidance of students and resident ophthalmologists in academic writing of papers and their participation in scientific events at local, national and international level.

- Involving and training students in research projects, focusing on teamwork.

-Training of resident ophthalmologists and other related specialties in research.

-Development of a feedback system to promote the optimization of knowledge transfer.

-Orientation of research towards efficiency and results with practical applicability, shortening treatment periods for the cure of ophthalmic disorders.

I.2. A cure in sight –researches regarding the tumors of the orbit, eye and ocular adnexa

I.2.1. Researches regarding on periocular carcinomas

Background

Eyelid carcinomas have the same histological and clinical features as malignant epithelial skin tumors and are a challenge in terms of plastic surgical reconstruction of large remaining eyelid defects (Tesluk, 1985; Lee *et al.*, 1999). Basal cell carcinoma develops from the basal layer of the epithelium and has several varieties; it can also develop on areas of pre-existing hyperkeratosis, frequently occurring on the scalp area as well, then on the nasolabial fold, eyelid, lower and upper lip skin, chin and forehead (da Cunha *et al.*,2015). Out of all skin cancer types, basal cell eyelid carcinomas are mostly frequent in clinical ophthalmic practice (Costea *et al*.,2019a). This type of carcinoma has the highest frequency out of all malignant eyelid tumors (Tesluk, 1985; Lee *et al*, 1999). Histologically, periocular basal cell carcinoma (pBCC) has been classified in twenty-six subtypes, described until now in literature (Wade and Ackerman, 1978). Basal cell eyelid carcinomas are classified as follows: nodular form, adenoids, basal squamous subtypes, which are low-risk tumors and the

morpheaform subtype, which has high risk (Grostern, 2003). In terms of frequency of eyelid skin tumour formation, after the basal cell carcinoma which is the most common, from others types like the squamous cell carcinoma, sebaceous carcinoma, Merkel cell carcinoma and skin melanoma (Youssef *et al.*,2010). Sebaceous gland carcinoma (SGC) of the eyelid is also called sebaceous gland adenocarcinoma, Meibomius or Zeis glands. The incidence of this tumor among all eyelid tumors varies between 2% and 7% and of all eyelid cancers between 1% and 5.5% (Kass and Hornblass,1989). Among the large studies, between 57% and 77% of afflicted patients were women (Chao *et al.*,2001; Kass and Hornblass,1989). Patients are usually between 50 and 90-year-old (Rao *et al.*,1982; Kass and Hornblass,1989). In most cases, the etiology of SGC is unknown, but a minority has a history of radiation exposure after retinoblastoma (Rao *et al.*,1982; Chao *et al.*,2001; Kass and Hornblass,1989). Basal cell carcinoma is the most common malignant tumor of the eyelid (1). It grows slowly in size and can cause local complications and aesthetic damage to the patient's eyelid (Hamada *et al.*,2005).

The most common location is in the upper eyelid and the most important risk factor is ultraviolet radiation (Hassain *et al.*,2011; Birt *et al.*,2004; Birt *et al.*,2007). Excision of large basal cell carcinoma involves reconstruction of the eyelid by various complex surgical techniques (Venkatesh *et al.*,2007). The treatment of these carcinomas is the most frequent surgically, their excision being performed within oncological limits, followed by the plastic reconstruction of the remaining defect (Costea *et al.*,2014). In terms of patients with very large eyelid carcinomas that invade the orbital tissue or metastasize to distant sites, treatment, radiotherapy combined with systemic chemotherapy are recommended (Saldanha *et al.*,2003). I had published research studies related to clinical, surgical and histopathological aspects regarding periocular carcinomas in the following papers:

Costea CF, Turliuc MD, Sava A, Dimitriu G, Dumitrescu GF, Dancă C, Cucu AI, Bogdănici CM, Costache II, Buzdugă CM, Ciocoiu M, Tănase DM, Dragomir RA, Cărăuleanu A. Periocular basal cell carcinoma: demographic, clinical, histological and immunohistochemical evaluation of a series of 39 cases. *Rom J Morphol Embryol*, 2019a, 60(1):77–86, IF=1.5.

Costea CF, Dimitriu G, Dumitrescu GF, Costache II, Sava A, Cucu A, Turliuc D. Surgical treatment in a case of lower eyelid basal cel carcinoma involving the ciliary margin, *Romanian Journal of Oral Rehabilitation*, 2014, 6(4): 99-103.

Costea CF, Petraru D, Dumitrescu G, Sava A. Sebaceous carcinoma of the eyelid: anatomoclinical data, *Romanian Journal of Morphology and Embryology*, 2013, 54(3): 665-668. IF=0.723

I.2.1.1. Researches regarding the correlations of the clinical and histological features of the periocular basal cell carcinomas

The clinical study was conducted retrospectively, on a number of 39 patients admitted to the Second Ophthalmology Clinic of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital Iași, Romania (from July 2007 to February 2018). We investigated demographic, clinical, histological and immunohistochemical (IHC) data of these patients clinically diagnosed with pBCC and histopathologically confirmed as pBCC. Specimens were processed and stained in the Pathology Laboratory of the same hospital with Hematoxylin-Eosin (HE) and IHC staining with the following anti-bodies: anti-cytokeratin (CK) AE1/AE3, CK5/6, K7, CK17, CK19, anti-CK20 and anti-epithelial membrane antigen (anti-EMA) (Table I.1) (Costea *et al*., 2019a).

Table I.1 – The antibodies used for IHC staining of pBCC and the expression pattern for the CKs and EMA in normal skin (Costea *et al*.,2019a)

Antibody	**Manufacturer**	**Clone**	**Antigen retrieval**	**Class**	**Dilution**	**Labeling**	**Cellular localization**
Anti-CK AE1/AE3	Thermo Fisher Scientific	AE1/AE3	Citrate, pH 6	Monoclonal Mouse antihuman CK AE1/AE3	RTU	Epithelial cells	Cytoplasmic
Anti-CK5/6	DAKO	D5/16 B4	Citrate, pH 6	Monoclonal mouse anti-human CK5/6	1:100	Epidermis (basal and parabasal cells), outer root sheath of hair follicle, glandular epithelia (sebaceous gland, sweat gland)	Cytoplasmic
Anti-CK7	DAKO	OV-TL 12/30	Citrate, pH 6	Monoclonal mouse anti-human CK7	1:100	Sebaceous gland (sebocytes and undifferentiated cells) and sweat glands (all parts except secretory cells)	Cytoplasmic
Anti-CK17	DAKO	E3	Citrate, pH 6	Monoclonal mouse anti-human CK17	1:40	Hair follicle (only in the bulge and follicular germinative cells), sebaceous gland (undifferentiated cells), and sweat gland (only in secretory cells)	Cytoplasmic
Anti-CK19	DAKO	RCK108	Citrate, pH 6	Monoclonal mouse anti-human CK19	1:100	Basal cells on the external root sheath of hair follicles) and sweat gland (only secretory cells)	Cytoplasmic
Anti-CK20	Thermo Fisher Scientific	EP23	Citrate, pH 6	Polyclonal rabbit antihuman CK20	RTU	Merkel cells from basal epidermal layer of the skin	Cytoplasmic
EMA	DAKO	E29	Citrate, pH 6	Monoclonal mouse anti-human EMA	1:50	Glandular and ductal epithelial cells of eccrine and apocrine sweat glands	Cell membrane and cytoplasmic

IHC: Immunohistochemical; pBCC: Periocular basal cell carcinoma; CK: Cytokeratin; EMA: Epithelial membrane antigen; RTU: Ready-to-use.

Results

For the 39 patients included in our retrospective study from 2007 to 2018, we identified the characteristics of pBCC of which are shown in Table I.2 (Costea *et al*.,2019a). The average age of patients at the time of surgery was 66 years (range:26-78 years), and out of these 43.6% were male with an average age of 64.84 years and 56.4% were female with an average age of 66.68 years. In the age group of 70-79 years were identified 13 cases (33.33%). Clinically the tumor was vegetating in 10 patients (25.64%) and was nodular-ulcerated in 29 patients (74.35%) (Table I.2, Figure I.1 a and b). Most patients had the pBCC localized in the lower eyelid (24 cases – 61.53%), then in the medial canthus (7 cases - 17.64%), in the upper eyelid in 5 cases (12.82%) and in 3 cases the exact location could not be specified (Costea *et al*.,2019a). Histologically, pBCC showed several subtypes: nodular subtype in 26 cases (66.7%) (Figure I.2, a and b; Figure I.3, a and b), adenoid subtype was identified in 4 cases (10.3%) and morpheaform subtype was diagnosed in one case (2.6%). pBCC with squamous differentiation was diagnosed in only eight patients (20.5%) (Table I.2) (Costea *et al*.,2019a). Correlating the sex of the patient with the histological subtype of the tumor, we found that nodular pBCC was almost in the same percentage identified in female and male patients (14 women versus 12 men). Morpheaform subtype affected only one man, and pBCC with squamous differentiation affected more female patients (5 females versus 3 males), and the adenoid subtype was identified in 3 female patients (Figure I.4).

Table I.2 – The characteristic features of the pBCCs from our series (Costea *et al*.,2019a)

Characteristics of the tumor	**Frequency No. (%)**
Localization	
Lower lid	24 (61.5%)
Medial canthus	7 (17.94%)
Upper lid	5 (12.82%)
Lateral canthus	-
Unspecified	3 (7.62%)
Side	
Right	18 (46.2%)
Left	21 (53.8%)
Gross pathology	
Vegetating lesion	10 (25.64%)
Nodulo-ulcerative (rodent ulcer)	29 (74.35%)
Histological Subtype	
Nodular	26 (66.7%)
With squamous differentiation	8 (20.5%)
Adenoid	4 (10.3%)
Morpheaform	1 (2.6%)

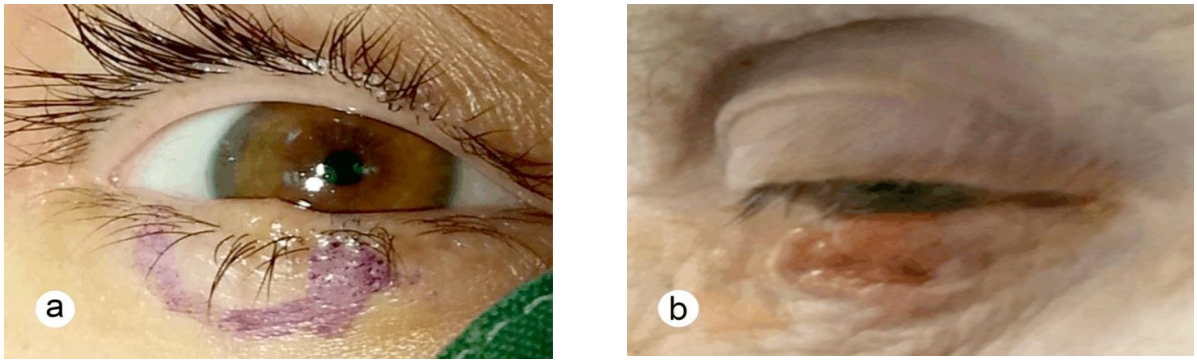

Figure I.1 – Clinical photo of pBCC: (a) Vegetating tumour; (b) Nodulo-ulcerative (rodent ulcer) tumour. pBCC: Periocular basal cell carcinoma (Costea *et al.*,2019a).

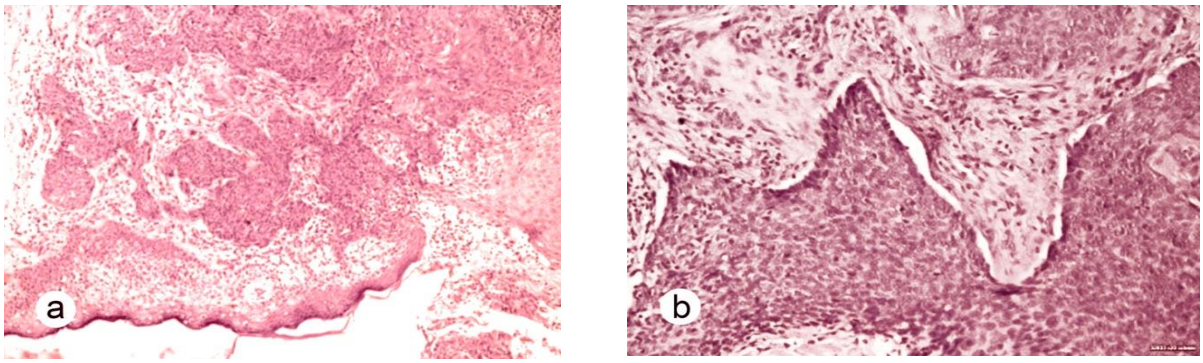

Figure I.2 – Nodular pBCC: (a) The tumour maintained epidermal connection, but invaded the dermis; (b) With higher magnification of the same case revealed tumoral masses consisting of basaloid cells, with peripheral palisading and artefactual clefts. HE staining: (a) ×100; (b) ×200. pBCC: Periocular basal cell carcinoma (Costea *et al*.,2019a).

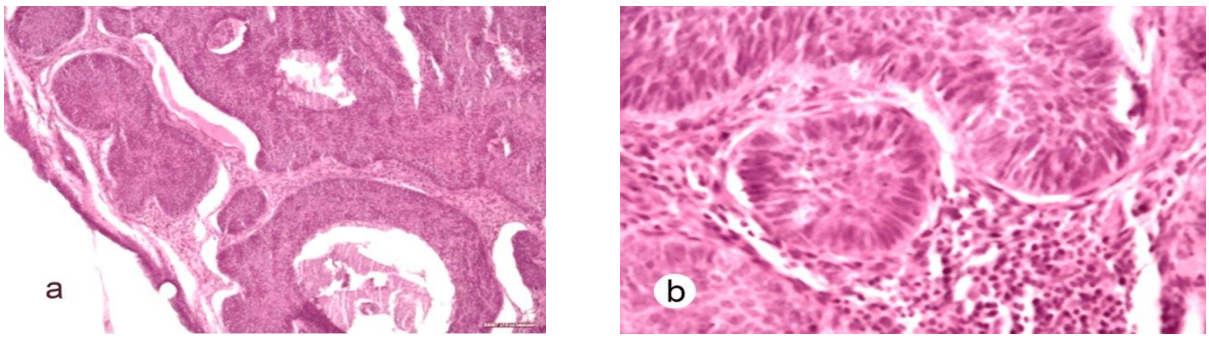

Figure I.3 – Nodular pBCC: (a) Basophilic tumoral nodules infiltrated the dermis; (b) The tumoral island presented a peripheral layer with a palisade arrangement, and was encircled by an artefactual retraction space between the tumour and the stroma – a strong chronic inflammation could be found in the tumoral stroma. HE staining: (a) ×100; (b) ×400. pBCC: Periocular basal cell carcinoma (Costea *et al*.,2019a).

The nodular subtype was found in the right eyelid in 12 patients, and the adenoid and squamous subtype also appeared in the right eyelid in an equal number of patients (3 patients) (Figure I.5.). The nodular subtype of pBCC was diagnosed in the left eyelid in 14 patients,

followed by the one with squamous differentiation in 5 patients in the right eyelid. In only one case of morpheaform basal cell carcinoma, it was located in the left lower eyelid (Costea *et al*.,2019a).

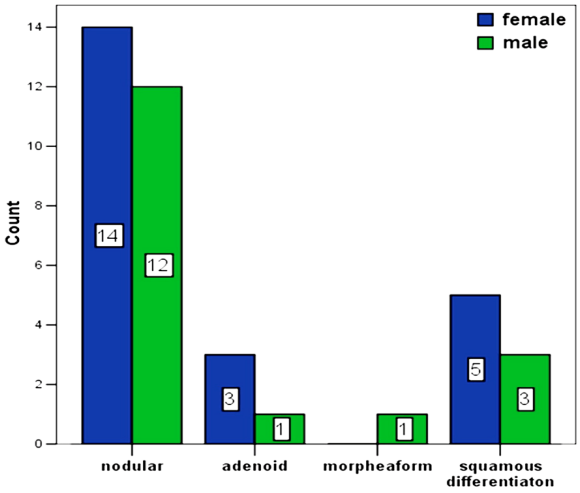

Figure I.4 – Distribution of pBCCs according to their histological subtypes (Costea *et al*.,2019a).

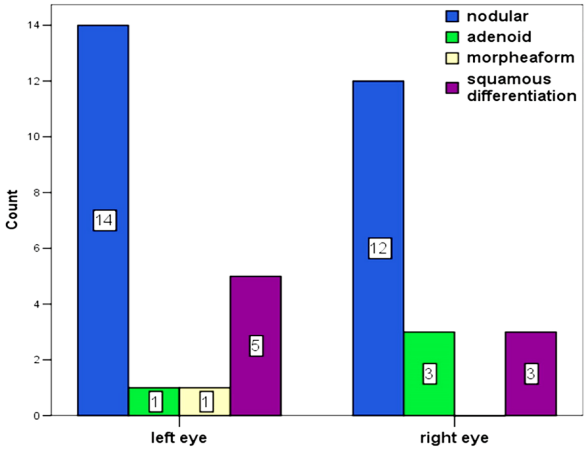

Figure I.5 – pBCC side location according to their histological subtypes (Costea *et al*.,2019a).

All 39 patients were clinically diagnosed by an ophthalmologist with an eyelid or canthus tumor; in only one case was the diagnosis of chalazion confirmed histopathologically, and in the rest of the cases the histopathological diagnosis was of pBCC.

The immunohistochemical profile of pBCC: strong-immuno-positivity for CK, AE1 / AE3 (Figures I.6 and I.7) and CK17 in all tumor subtypes studied. CK 5/6 was positive in the areas of squamous differentiation of pBCC (Costea *et al.*,2019a). In all cases of pBCC, there was no immunopositivity for CK , CK19 (Figure I.8, a and b), CK20, and EMA (Figure I.9, a and b).

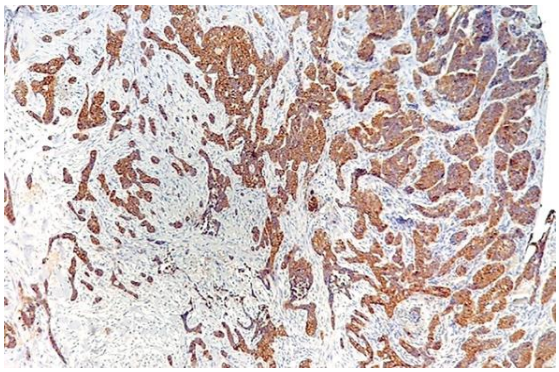

Figure I.6 – pBCC with very intense CK AE1/AE3 expression. Anti-CK AE1/AE3 antibody immunostaining, ×100 (*Costea et al*.,2019a).

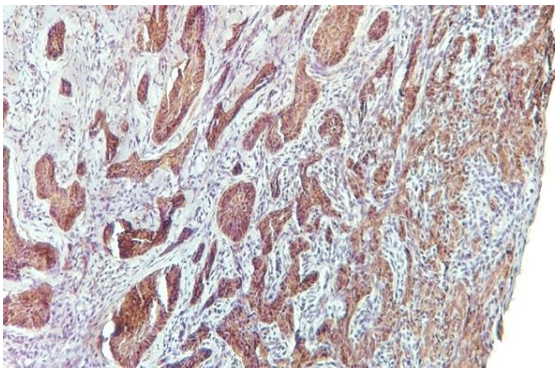

Figure I.7 – pBCC showing strong immunopositivity for CK AE1/AE3. Anti- CK AE1/AE3 antibody immunostaining,×200 (Costea *et al*.,2019a).

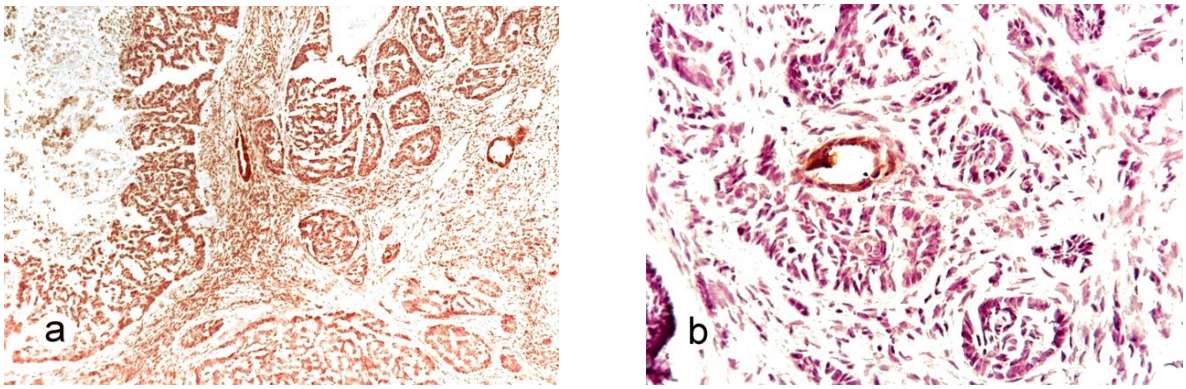

Figure I.8 – Strong CK19 immunopositivity of intratumoral remnants of secretory cells of sweat glands, but there was no staining of pBCC. Anti-CK19 antibody immunostaining: (a) × 100; (b) ×400. CK: Cytokeratin; pBCC: Periocular basal cell carcinoma (Costea *et al*.,2019a).

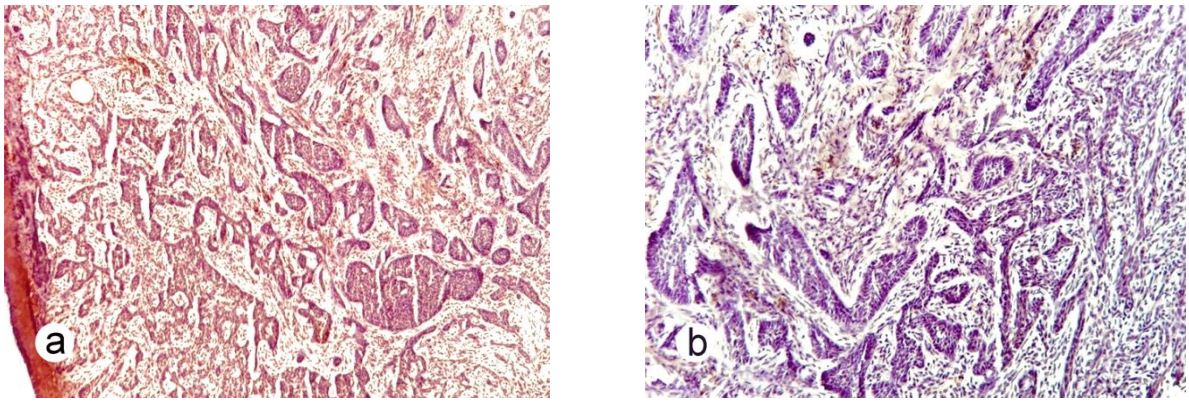

Figure I.9 – pBCCs did not express immunoreactivity with anti-EMA antibody immunostaining:(a) ×100; (b) ×200. pBCC: Periocular basal cell carcinoma; EMA: Epithelial membrane antigen (Costea *et al*.,2019a).

Discussions

The clinical diagnosis of pBCC is based on the patient's history and ophthalmological examination, being confirmed by the anatomo-pathological diagnosis of the surgical resection specimens (Costea *et al*.,2019a). The prevalence of these pBCC has been reported in the literature as being higher in male patients (Wu *et al*., 2014). In 2017, however, Hui *et al*. found a higher prevalence in female patients (Hui *et al*., 2017), as in our study. In this study, after surgical excision of pBCC established that the histopathological diagnosis nodulo-ulcerative subtypes were more common (Costea *et al*.,2019a).

Furdova *et al*., mentioned in the study published in 2015, that the most common are nodular cell carcinomas (Furdova *et al*., 2015) and in our study we found a percentage of 66.7% of patients with this subtype of pBCC (Costea *et al*.,2019a). One case of morpheoform pBCC was identified in the lower left eyelid, and pBCC with squamous differentiation was also found, in the lower left eyelid, this location being associated with the most aggressive

subtypes (Costea *et al*.,2019a). In 2015, Bălășoiu *et al*., in a similar study, found that the nodular form of pBCC occurs in 70% of cases, the adenoid form in almost 20%, the cystic form in 12.5% and the morpheoform form in 10% of patients (Bălășoiu *et al*., 2015). Examination of pBCC from a clinical point of view is essential, highlighting whether it is an ulcerated or a nodular form, whether it changes the appearance of the eyelid and whether it involves loco-regional lymph nodes. Clinical studies published to date in the literature show that these pBCC are frequently located in the lower eyelid (52.7-63.1%), followed by the medial canthus (29.8%), the upper eyelid (7.5 - 5.7%) and the lateral canthus (2.9 - 1.4%) (Lee *et al.,* 1999; Wu *et al*., 2017; Pornpanich and Chindasub 2005; Spraul *et al.,* 2000), data that are consistent with our study (Costea *et al.,*2019a). Some authors reported that pBCC develops more frequently on the right side, but others reported a higher incidence on the left side, as we showed also in our clinical study (Furdova *et al.,* 2015; Costea *et al.,*2019a).

Clinically pBCC has a nodular-ulcerative appearance, with a slow, painless clinical course of at least three years (Mallajosyula, 2008). Many studies searched for an IHC panel to distinguish basaloid carcinoma from the squamous cell carcinoma. In our study we found that all histopathological subtypes of pBCC expressed strong immunopositivity for anti-CK AE1/AE3 antibody (Costea *et al*.,2019a). Other authors obtained the same results (Cuevas-González *et al.,* 2016). Squamous cell carcinoma expressed in more than 80% of cases a strong positivity for this marker and other studies found that the pBCC showed no staining for EMA antibody (Ramezani *et al*., 2016). We also noted in our study that EMA has no expression in pBCC (Costea *et al.,*2019a). These findings help distinguish between basal and squamous cell carcinoma of the eyelid skin by routine immunohistochemistry using anti-EMA antibody (Beer *et al.,* 2000). In 2012, Andersson *et al.* revealed that CK17 is a very important marker in the identification of pBCC, especially the morpheaform subtype pBCC that must be distinguished from other adnexal neoplasms, for example desmoplastic trichoepithelioma (Anderson-Dockter *et al*., 2012). Our research proved that anti-CK17 antibody is a reliable marker also for pBCC as we obtained a strong immunopositivity for our cases (Costea *et al.,*2019a). In our study, pBCC did not express CK19 positivity. With this antibody, we identified only remnants of sweat glands entrapped into the tumors (Costea *et al*.,2019a). As such, pBCC seems to have its origin in UV light induced mutations on outer sheath of the hair follicle cells as some other authors have also pointed out (Alessi *et al*.,2008). In our study, we showed that the CK profile of pBCC consisted in CK AE1/AE3 and CK17 intense immuno-positivity, and CK5/6 immunopositivity only in squamous

differentiation of pBCC. All the other CKs (CK7, CK19, CK20), and also EMA did not express immunopositivity in our specimens (Costea *et al*.,2019a). Morpheaform and adenoid types also presented 20% expression of Ki67 labelling index, while the cystic type presented Ki67 expression in less than 10% of the malignant cells nuclei (Costea *et al*.,2019a).

Conclusions

Our study found that most pBCC develop in elderly patients. These tumors are identified in the lower eyelid preferably on the left side, and most cases are classified as nodular subtype of pBCC. As an immunohistochemical profile, there is a strong positivity for CK17, although the origin of these carcinomas is in follicular germ cells. The results of our study showed IHC profile, clinical, histological features which seems to be representative for Eastern and Central European countries, maybe due to the same environmental factors and genetic predisposition (Costea *et al.*,2019a).

I.2.1.2. Surgical aspects of basal cell carcinoma of the eyelids: a clinical case study

Background

Basal cell carcinoma is the most common malignant tumor of the eyelid (1). It grows slowly in size and can cause local complications and aesthetic damage to the patient's eyelid (Hamada *et al*.,2005). The most common location is in the upper eyelid and the most important risk factor is ultraviolet radiation (Hassain *et al*.,2011; Birt *et al*.,2004; Birt *et al*.,2007). Excision of large basal cell carcinoma involves reconstruction of the eyelid by various complex surgical techniques (Venkatesh *et al.*,2007). I published a case of basal cell carcinoma of the eyelid in which I reconstructed the lower eyelid using the Hughes technique. The data on this case have been published in an indexed journal in the international database and are set out below.

Clinical Case of an eyelid basal cell carcinoma **-** An 85-year-old female patient was hospitalized in the Second Ophthalmology Clinic of the "Prof.Dr. Nicolae Oblu "Emergency Clinical Hospital of Iași, Romania, for a tumor located in the lower eyelid, which grew in size for 2 years. Clinical ophthalmologic examination revealed a vegetative and ulcerated tumor that infiltrated the ciliary margin, about 8/6 mm in diameter, located in the middle 1/3rd of the left lower eyelid (Figure I.10). Tumor excision was performed with a 2 mm safety margin in healthy tissue, then the lower eyelid was reconstructed using the Hughes technique. The surgical technique consisted of the rectangular excision of the full eyelid tchicness area containing the tumor (Figure I.11), harvesting a tarsoconjunctival pedicle flap from the upper eyelid (Figure I.12), suture of the tarsoconjunctival flap from the upper eyelid to the deep

plane of the lower eyelid defect (Figure I.13), harvesting a free skin graft larger than the defect, from the retroauricular region (Figure I.14) and suture of the free skin graft to the superficial lower eyelid defect (Figure I.15).

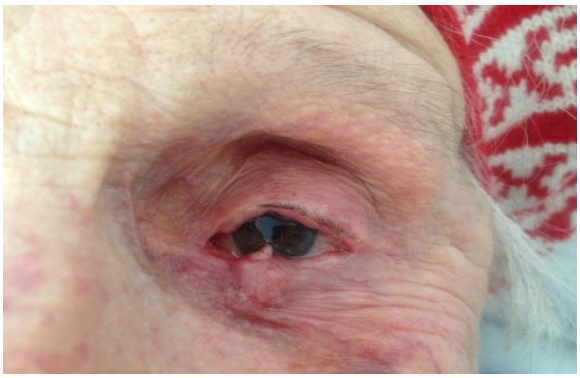

Figure I.10 - The patient photography:a vegetative and ulcerated tumor located in the medial 1/3rd of the lower eyelid, which involves the cilliary margin (Costea *et al*., 2014).

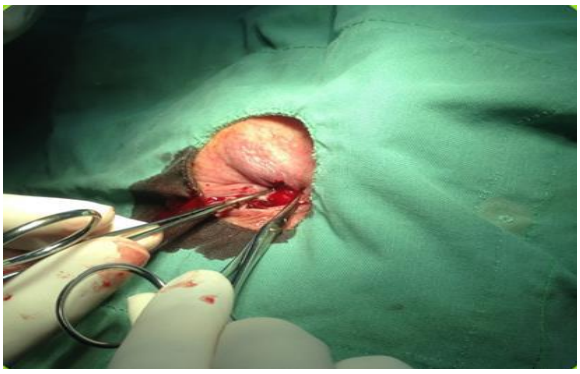

Figure I.11 - Rectangular excision of the tumor (Costea *et al*., 2014).

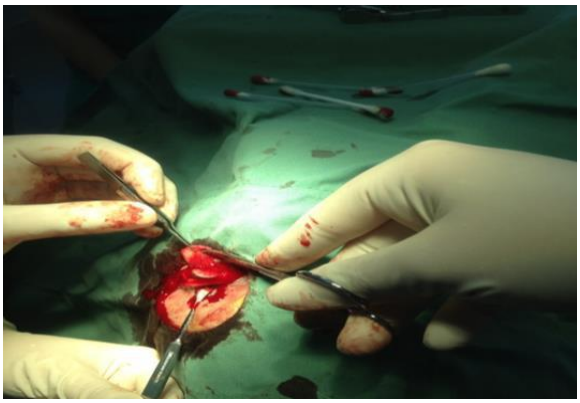

Figure I.12 - Excision of a tarsoconjunctival pedicle flap from the upper eyelid at 4 mm above eyelid margin (Costea *et al*., 2014).

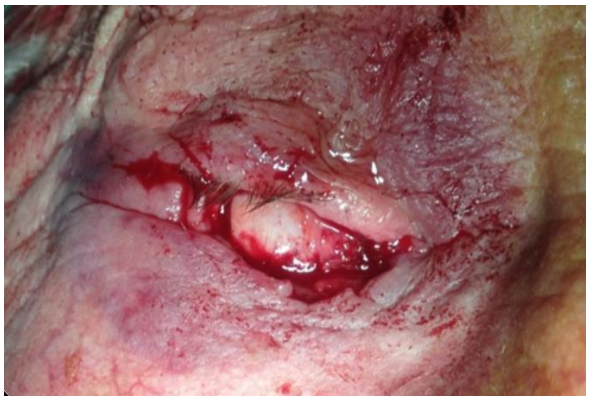

Figure I.13 - Suture of the tarsoconjunctival flap to the lower eyelid defect (Costea *et al*., 2014).

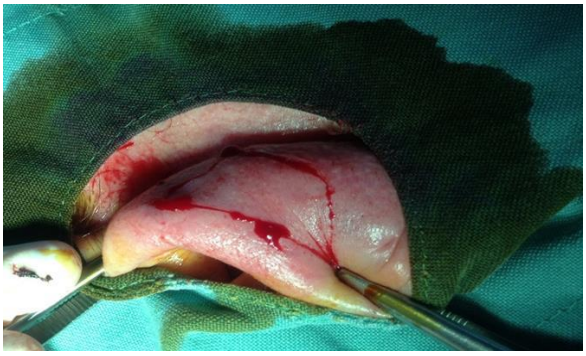

Figure I.14 - Harvesting a free skin graft from the retroauricular region (Costea *et al*., 2014).

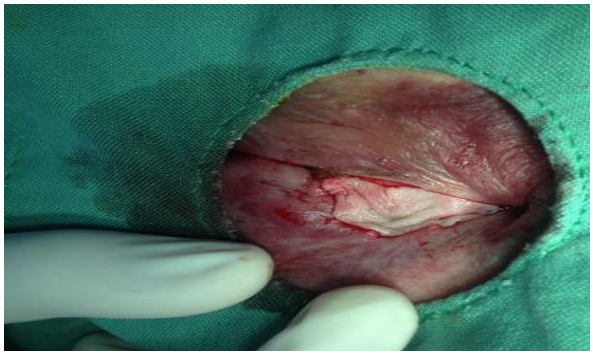

Figure I.15 - Suture of the free skin graft to the superficial plane of lower eyelid defect (Costea *et al*., 2014).

The postoperative course was favorable. After 3 weeks the flap was transected to create a palpebral fissure (Figure I.16 and I.17) (Costea *et al*.,2014). Histopathological examination of the specimen revealed an ulcerated basal cell carcinoma that originated in the basal layer of the epidermis and invaded the eyelid with "nests" and "islands" of basaloid tumor cells with hyperchromatic nuclei (Costea *et al*., 2014).

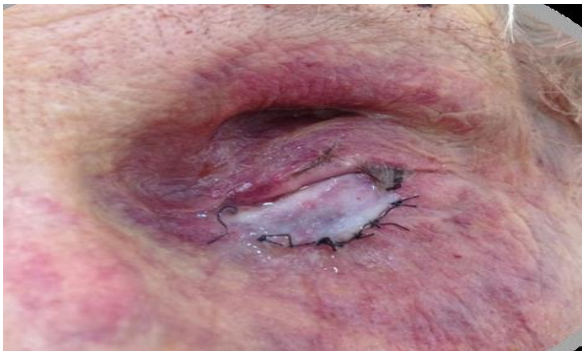

Figure I.16- Post operative results at first day (Costea *et al*., 2014).

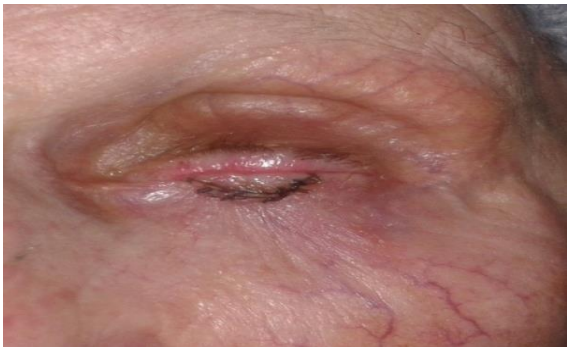

Figure I.17 - The patient photography: 3 weeks after surgery (Costea *et al*., 2014).

Discussions

Surgical treatment of eyelids carcinomas involves simple excision of the tumor with a safety margin between 3 and 5 mm, followed by reconstruction of the eyelid in certain situations.The principles of surgical reconstruction of the eyelids depend on the size and depth of the defect, the inclusion of the internal or external canthus or the involvement of the lacrimal system (Hayano *et al*.,2012).Defects that are small can be closed directly. Defects whose size varies between 30-50% require eyelid plastic surgery techniques: lateral cantotomy, cantolysis, sliding plasty and Tenzel semicircular advancement flap. In the case of defects larger than 50% of the lower eyelid, the Hughes technique is applied and those located on the upper eyelid require the Cutler-Beard flap technique (Subramanian,2011). In our patient the tumor was located in the middle 1/3rd of the lower eyelid and involved over 50% of its length. The loss of tissue following the excision, of the lower eyelid tumor (with 2 mm safety margins) required the restoration of the deep tarsoconjunctival plane with a tarsoconjunctival flap from the upper eyelid and of the superficial plane with a free skin graft from the retroauricular region, according to Hughes technique (Costea *et al*., 2014).

Conclusions

In large tumors located on the eyelids we recommend that the size of the resulting surgical excision defect to be measured and adequate techniques for eyelid plasty to be used

for preserving the normal appearance and function of the eyelids. A surgical plan tailored to each individual patient will ensure complete healing without recurrences and other postoperative complications (Costea *et al*.,2014).

I.2.1.3. Anatomical and clinical data on sebaceous gland carcinoma of the eyelid

Background

Sebaceous gland carcinoma (SGC) of the eyelid is a rare, very aggressive, slow-growing malignant tumor that can often be confused with other inflammatory lesions of the eyelids (Costea *et al*., 2013). SGC of the eyelid is also called sebaceous gland adenocarcinoma, Meibomius or Zeis glands. The incidence of this tumor among all eyelid tumors varies between 2% and 7% and of all eyelid cancers between 1% and 5.5% (Kass and Hornblass,1989). Among the large studies, between 57% and 77% of afflicted patients were women (Chao *et al.*,2001; Kass and Hornblass,1989). Patients are usually between 50 and 90-year-old (Rao *et al.*,1982; Kass and Hornblass,1989). In most cases, the etiology of SGC is unknown, but a minority has a history of radiation exposure after retinoblastoma (Rao *et al*.,1982; Chao *et al*.,2001; Kass and Hornblass,1989). Clinically, SGC is characterized by the "masquerade syndrome" of some benign and malignant tumors, resulting in the delay of correct and early diagnosis. For these reasons, ophthalmologists, dermatologists and other specialists should be familiar with the clinical features and appropriate treatment of these tumors (Rao *et al.*,1982; Kass and Hornblass,1989). Early and correct diagnosis of this type of carcinoma is essential in preventing local recurrence and metastasis (Rao *et al*.,1982; Chao *et al*.,2001; Esmaeli *et al.*,2012).

Clinical case of a sebaceous gland carcinoma of the eyelid

The clinical case presented is of a female patient, aged 78, hospitalized in 2013 in the Second Ophthalmology Clinic of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania. At the clinical examination, a tumor was found that formed a year ago, but which has grown in size in the last 6 months. The patient had previously been treated medicinally and surgically for the same formation, but was diagnosed with recurrent chalazion in the right upper eyelid.Following surgical excision with oncological safety margins, the histopathological diagnosis was sebaceous gland carcinoma of the eyelid (Costea *et al.,* 2013). The specimens were fixed in the Pathology Laboratory of the same hospital according to the classical techniques in paraffin blocks, sectioned and stained with Hematoxylin-Eosin (HE), then immunohistochemical reactions (IHC) were performed for epithelial membrane antigen and Ki-67 (Costea *et al*.,2013).

Results

The ophthalmological examination revealed a nodular, ulcerated formation about 1 cm in diameter located in the middle 1/3rd of the right upper eyelid, immobile on the adjacent tissues; no local lymphadenopathy was found (Figure I.16) (Costea *et al*.,2013). The surgically excised mass was composed of tumor lobules surrounded by a vascular-connective stroma infiltrating the dermis and presenting central cores of necrosis having a comedocarcinoma-like pattern (Figure I.17). In some lobules, tumor cells showed varying degrees of sebaceous differentiation, with foamy cytoplasm and fine vacuoles. The centrally located nuclei were hyperchromatic, pleomorphic, with conspicuous nucleoli and presented atypical mitosis (Figure I.18).

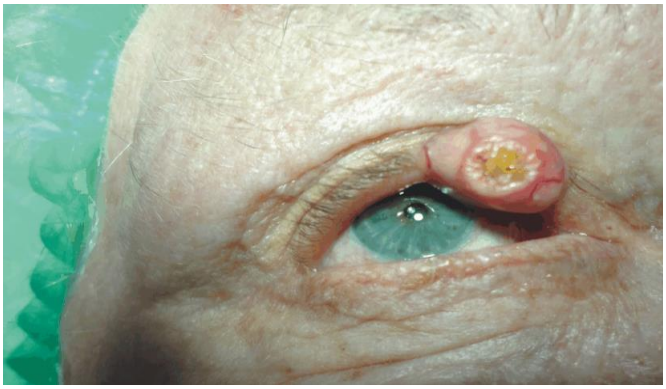

Figure I.16 – Sebaceous gland carcinoma of the right upper eyelid. Firm, yellow tumor nodule with central ulceration (Costea *et al*.,2013).

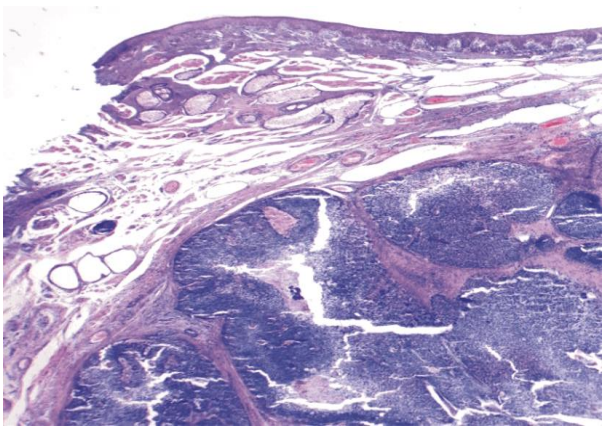

Figure I.17 – Sebaceous carcinoma with lobules infiltrating the dermis, composed of tumor cells with basaloid differentiation, and comedocarcinoma - like pattern (HE stain, ×40) (Costea *et al*.,2013).

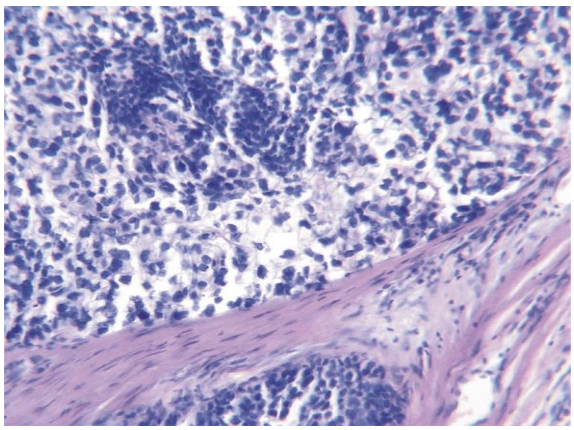

Figure I.18 – Sebaceous carcinoma composed of tumor cells showing sebaceous differentiation (HE stain, ×200) (Costea *et al*.,2013).

The neoplastic lobules showed basaloid differentiation and squamous differentiation with keratinization and forming of corneous globes. Thirty-three percent of tumor cells were positive for Ki-67, consistent with a high-proliferative activity of tumor cells. Immunohistochemical staining for EMA was intensely positive in most tumor cells. Given the degree of sebaceous differentiation, this tumor was classified as sebaceous carcinomas with basaloid and squamous areas (Costea *et al*.,2013).

Discussions

The first case of periorbital SGC was published by Allaire, in 1891 (Natarajan *et al*.,2011). SGC of the eyelid are rare tumors originating in the meibomian glands, Zeis glands, or both when of multicentric origin. In some cases, the tumor origin cannot be determined (Kan *et al*.,2011; Dasgupta,2009; Kale *et al*,.2011).SGC of the eyelid occurs in people aged 50 to 90 years but it can also be seen in young people (Kass *et al.,* 1989; Natarajan *et al.,* 2011; Kan *et al*.,2011; Dasgupta *et al*.,2009). Some studies suggest a female preponderance, while others show no gender preference (Rao *et al*.,1982; Kass *et al.*,1989; Shields *et al.*,2005; Dasgupta *et al.*,2009). From a clinical point of view, SGC has the appearance of a yellow nodule, small in size, hard in consistency, which can be misdiagnosed, most often as a chalazion, as in the case of the patient presented by us (Costea *et al*.,2013). SGC of the eyelid is known for masquerading as a benign or malignant condition ("masquerade syndrome") often causing a delay before correct diagnosis. The misleading clinical manifestations may suggest an inflammation, including unilateral conjunctivitis, blepharitis, blepharocojunctivitis and keratoconjunctivitis (Dasgupta *et al*.,2009; Shields *et al*.,2004).The patient was treated medicinally and surgically for chalazion for several months, so the tumor recurred and also grew in size up to 10 mm in diameter (Costea *et al*., 2013). The size of the tumor has an important prognostic role, so for tumors with sizes between 6-10 mm, mortality varies between 18% and 60% when the tumor is between 11-20 mm mortality increases (Rao *et al.*,1982). Microscopically, the tumor diagnosed by us had the appearance of a moderately differentiated sebaceous carcinoma with basaloid and squamous areas (Costea *et al*.,2013). Studies reveal erroneous diagnosis in 23–77% of the poorly differentiated sebaceous carcinomas (Shields *et al*.,2004). In about 18% of cases, sebaceous carcinoma may be mistaken for squamous cell carcinoma (Shields *et al*.,2004). SGC of the eyelid should be differentiated from basal cell and squamous cell carcinoma, so Sinard JH (1999) used immunohistochemical reactions, finding that it generally expresses EMA, Cam 5.2 and BRST-1, while basal cell carcinoma does not express either EMA or BRST- 1, and

squamous cell carcinoma expresses EMA, but not Cam 5.2. (Sinard, 1999).

In our case, the tumor cells showed an intensely positive reaction to EMA and an increased proliferative activity, confirmed by an increased Ki-67 index (Costea *et al*., 2013).The study, published by Rao N.A. *et al.*, showed that localization in the upper eyelid and tumor size larger than 10 mm but also the duration of symptoms longer than five months indicates an unfavorable prognosis (Rao *et al*.,1982). In our case, although all the clinical aspects place us in the situation described above, we did not find the invasion of the local lymph nodes or distant metastases 5 months after the operation (Costea *et al*.,2013). Local excision of the tumor, orbital exenteration, radiation therapy and chemotherapy are the methods used in the management of patients with SGC of the eyelid in different clinical stages (Kale *et al*.,2012; Hata *et al*.,2012).

Conclusions

SGC is a rare tumor located in the eyelids, which can often be confused with other inflammatory lesions of the eyelids. Early and correct clinical diagnosis, surgical excision of the formation within oncological safety limits, prevents mortality and morbidity in these patients (Costea *et al*.,2013).

I.2.2. Researches regarding the clinical and histopathologic features of the cutaneous horn of the eyelids

Background

The cutaneous horn (cornu cutaneum) is a circumscribed, conical and keratotic lesion, which can hide benign or malignant lesions (Mantese *et al*.,2010; Thappa *et al*.,2004; Copcu *et al*.,2004). The clinical diagnosis is set based on its appearance, the lesion being classified as solitary or multiple, straight, curved or twisted, white or yellow (Michal *et al*.,2002; Nthumba *et al*.,2007), most often located at the level of the skin on the patient's face (Michal *et al*.,2002; Duncan *et al*.,2003; Souza *et al.*,2003). While the cause leading to the formation of cutaneous horns (Bondeson, 2001) is unknown, UV radiations are believed to be the trigger of this condition (Padmini, 2015). Usually, the cutaneous horn occurs in people over the age of 50, in both genders (Bart *et al*.,1968; Yu *et al.*,1991; Feste *et al.*,1995; Castillo *et al*,2002; Rush *et al*.,2015). This benign tumor develops on benign lesions (verrucous epidermal nevus, seborrheic keratosis, viral warts, moluscum contagiosum, inverted follicular keratosis, Bowen's disease, solar keratosis, arsenical keratosis) and malignant lesions (basal cell carcinoma, squamous cell carcinoma, metastatic renal carcinoma, sebaceous carcinoma, granular cell carcinoma or Kaposi sarcoma) (Thappa *et al*.,2004; Copcu *et al*.,2004; Rush *et*

al.,2015).

I published an article highlighting the anatomoclinical features of the cutaneous horn of the eyelid, to highlight the pre-existing lesions on which this benign tumor is formed and the most important data are in the followings.

I presented two clinical cases of cutaneous horn of the eyelid diagnosed in two patients aged 19 and 78 years, which develop on pre-existing skin lesions: chalazion and inclusion cyst associated with epithelial dysplasia in both cases.

Costea CF, Dimitriu G, Sava A, Chihaia M, Danca C, Cucu A, Dumitrescu N, Turliuc D. Cutaneous horn of the eyelid: anatomoclinical implication, *Journal of Clinical Research and Ophthalmology*, 2017a, 4(1): 1-5.

Clinical Case No.1

A 19-year-old male patient, resident in a the rural area, was admitted to the 2nd Ophthalmology Clinic of the "Prof.Dr.Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania, for a solitary firm horn on the right lower eyelid, which had gradually progressed over the course of two months. One year before, the patient noticed a focal swelling of the inferior right eyelid treated empirically, on which the cone shape growth developed progressively.

The patient's medical and ocular history was not significant. The clinical examination revealed a solitary cone shape hyperkeratotic growth measuring 1.0/0.6 cm in size, with an inflamed nodular base, located in the middle 1/3rd of the inferior right eyelid (Figure I.19).

There was no regional lymphadenopathy. The clinical diagnosis was that of solitary inferior right eyelid cutaneous horn. The lesion was excised completely with local anesthesia, and the defect was closed by sliding the skin of the inferior eyelid and sutured with 6.0 Vicryl.

The resection specimen was evaluated histologically, revealing an association of three vertically overlapped lesions: compact acellular keratin, with a "dome" shape (Figure I.20), overlying an hyperplastic epithelium showing an infection with human papilloma virus and a moderate dysplasia of the adjacent epidermis.

Underneath the hyperkeratosis and the hyperplastic epidermis, a chalazion could be identified into the deep dermal structure.

The postoperative evolution was favorable: no scar formation and no clinical relapse for six months (Costea *et al*.,2017a).

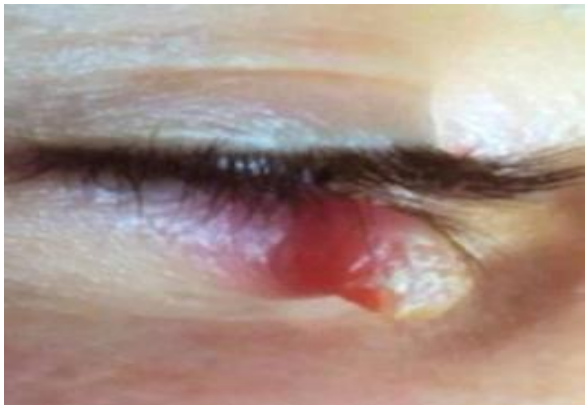

Figure I.19 - Right lower eyelid cutaneous horn (19-year-old patient) (Costea *et al.*,2017a).

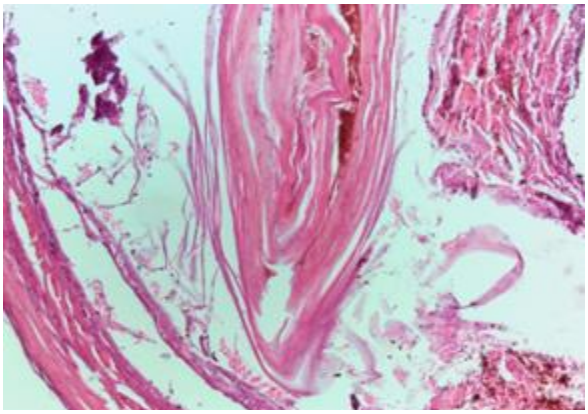

Figure I.20- Microscopic examination: acellular keratin layers, displayed in Figure 2: Microscopic examination: acellular keratin layers, displayed in a "dome" fashion (black arrow) (Hematoxylin-Eosin staining, x10 original magnification) (Costea *et al.*,2017a).

Clinical Case No.2

The second case is a 78-year-old male patient from a rural area, hospitalized in the Second Ophthalmology Clinic of the "Prof.Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania. It had a solid consistency conical tumor on the right upper eyelid, which grew in size over 6 months.

Clinical ophthalmologic examination revealed a hyperkeratosis conical tumor formation, which had the appearance of an inflammatory nodule, with ulcerated edges 0.6 / 0.3 cm in size, developed in the right upper eyelid (Figure I.21).

The patient had numerous pigmented lesions on his face skin. Biomicroscopic examination revealed an incipient cataract in both eyes. There was no loco-regional lymphadenopathy.

The clinical diagnosis was a cutaneous horn located on a sebaceous cyst in the right upper eyelid.

The tumor was completely excised with oncological safety margins. The histopathologic exam revealing an association of three lesions: cutaneous horn (which "detached" from the basis of the underlying lesion, just before the surgical intervention), moderate dysplasia of the underlying epidermis and epidermal inclusion cyst located in the deep dermal structure (Figure I.22). The postoperative evolution was favorable: no scar formation and no clinical relapse for a year (Costea *et al.*,2017a).

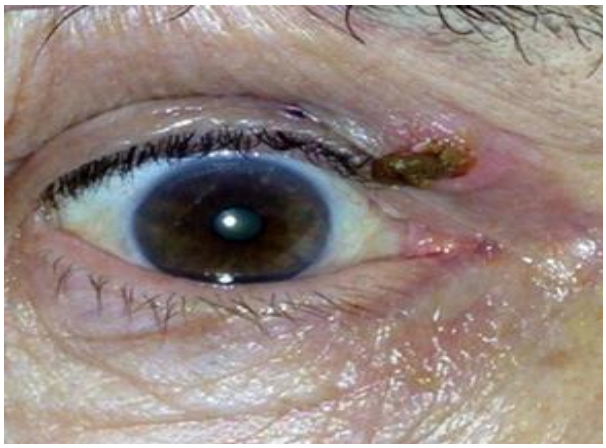

Figure I.21 - Right upper eyelid cutaneous horn (78-year-old patient) (Costea *et al.*,2017a).

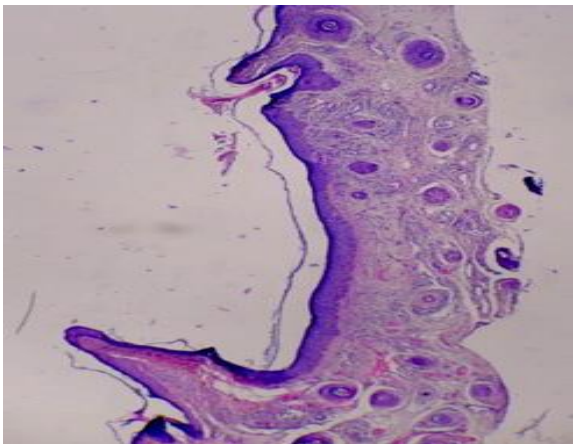

Figure I.22 - The palpebral cutaneous fragment presenting two epidermal projections located at a distance, between which a depression is delimited (black arrow). In that area, the epidermis showed hyperkeratinization, remnant of the cutaneous horn which detached very easily just before the surgical intervention (Hematoxylin Eosin staining, x100 original magnification) (Costea *et al.*,2017a).

Discussions

The cutaneous horn is a clinical diagnosis which refers to a tumor located on the surface of the skin (Rush *et al*.,2015; Durkee,1866) with a hyperkeratotic cone shape (Copcu *et al*.,2004), white-yellowish in color, ranging from a few millimeters to a few centimeters in size, which can hide other benign or malignant underlying lesions (Mantese *et al*.,2010). The cutaneous horn is made of compact keratin. The basis can be flat, nodular or „crater-like". Clinical aspects cannot give any clue to differentiate a benign or a malignant lesion, but the indurated and bleeding base of a large tumor pleads in favour of malignancy (Rush *et al.*,2015). From a histological perspective, the cutaneous horn is made of compact hyperkeratosis, which can be either orthokeratotic (an anuclear keratin layer, as in the normal epidermis), (The Free Dictionary, orthokeratosis, 2012) or parakeratotic (retention of nuclei in the cells of the stratum corneum of the epidermis), (The Free Dictionary, Parakeratosis, 2012). Moreover, it is often associated with acanthosis; the base of the cutaneous horn will highlight a pathological process which is responsible for its formation (Mantase *et al*., 2010; Yu *et al*.,1991; Rush *et al*., 2015). The most common types of lesions that can be identified histopathologically at the base of the cutaneous horn include: actinic keratosis, keratoacanthoma, seborrheic keratosis, pyogenic granuloma, discoid lupus erythematosus,

verruca vulgaris, epidermal nevus, trichilemmal cyst, trichilemmoma, prurigo nodules, intradermal nevi, Bowen's disease, basal cell carcinoma and squamous cell carcinoma (Rush *et al*., 2015; Vano-Galvan *et al*.,2008; Cruz Guerra *et al.*,2005; Mencía-Gutiérrez *et al*.,2004). In the first case presented by the 19-year-old patient, the lesion was found to have developed on a chalazion, but the adjacent epidermal showed moderate dysplasia, requiring a wider surgical excision in healthy tissue (Costea *et al*., 2017a). In the second case, the patient came from rural areas, being exposed to solar radiation and the skin horn developed on an epidermal inclusion cyst, but also in this adjacent epidermal case showed moderate dysplasia, which also required extensive surgical excision of the base of the cutaneous horn (Costea *et al*., 2017a).

In these two cases there is an association of benign lesions and there are also present premalignant lesions. The clinical appearance of the cutaneous horn may mask the premalignant or malignant lesion of the underlying lesion. Therefore, a wide excision of the base of the lesion in healthy tissue is required (Costea *et al.*,2017a). However, if an association of the cutaneous horn with a malignant lesion is found, the patient must be assessed in order to diagnose potential relapse. In the case of patients with squamous cell or basal cell carcinomas at the base of the cutaneous horn, a screening must be performed to prevent relapses every 3-12 months for first 2 years, every 6-12 months for 3 years, and then at least annually for life (National Comprehensive Cancer Network,2016).

Conclusions

The cutaneous horn is a lesion which, from a clinical perspective, cannot give the physician any indication as to its nature, i.e. benign or malignant; it can mask several benign, premalignant, as well as malignant lesions, and can only be diagnosed through a histopathological examination, which is essential in later therapeutic conduct (Costea *et al.*, 2017a).

I.2.3. Researches regarding the clinico-pathological aspects of juvenile compound conjunctival nevi of the eye

Background

Conjunctival nevi affect children and adolescents, being rare lesions, located in the eye conjunctiva. These account for 2.5% of all benign conjunctival lesions (Novais *et al*.,2010). Most nevi are detected at the age of puberty, when there is a proliferation of nevi cells, favored by hormonal changes (Shields *et al*.,2004; Shields *et al*., 2007; Shields et al.,2006; Keijser *et al*.,2007; Tananuvat *et al*.,2008; Nolan *et al*.,2001; Barros *et al*.,2009),

but can be found at any age, even in the elderly (Albreiki *et al*.,2012). The most common melanocytic lesions of the conjunctiva are congenital nevi, which are present at birth in 5% of cases, or may occur in the first 6 months of life, when they are acquired (Novais *et al.*,2010; Shields *et al.*,2004). Among the conjunctival hyperpigmented lesions, nevi are found in 1/3rd of them, and in 10% of cases malignant melanoma is diagnosed (Novais *et al*.,2010; Alkatan *et al.*,2010). There are few publications in the literature on juvenile compound conjunctival nevus, the earliest mention of the conjunctival nevus was in 1965 by Barrie Samuel Jay (1929-2007) who reported the pathological aspects of conjunctival nevus (Jay,1965). Juvenile conjunctival nevus have various names in literature: inflamed juvenile conjunctival nevus (Levi-Schaffer *et al*., 2002), inflamed conjunctival nevus of puberty (Folberg *et al.*,1989) or inflammatory juvenile conjunctival nevus (IJCN), (Lommatzsh *et al*.,2007).

The aim of my study was to present the clinic-pathological aspects of the IJCN and to highlight its particular, clinical and histopathological characteristics and it was published in an ISI ranked journal.

Costea CF, Turliuc DM, Dimitriu G, Bogdanici CM, Anca Motoc, Chihaia MA, Dancă C, Cucu A, Cărăuleanu A, Dumitrescu N, Indrei L, Turliuc S. Inflammatory juvenile compound conjunctival nevi. A clincopathological study and literature review, *Romanian Journal of Morphology and Embryology*, 2017c, 58(3): 739-747, IF=0.912.

We study two cases of IJCN, which were admitted in the 2nd Ophthalmology Clinic "Prof.Dr.Nicolae Oblu" Emergency Clinical Hospital, Iași, România over a period of five years (from Yuly 1, 2012, to June 30, 2017).

Clinical Case No.1

A 13-year-old male patient was admitted in the 2nd Ophthalmology Clinic, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania in 2017. The patient complained having a lesion on the right eye conjunctiva, which grew in size for 14 months, and was accompanied by irritative symptoms.

Clinical examination with slit lamp biomicroscopy revealed a single, not well-defined, slightly elevated, pinkish, movable plaque of 7 mm at its widest point, with vascular congestion. It was located in the bulbar conjunctiva, 1 mm close to the temporal limbus (Figure I.23).

The patient had been diagnosed before with recurrent allergic conjunctivitis and with mitral and tricuspid congenital regurgitation of degree I–II (Costea *et al.*,2017c). The

conjunctival tumor was excised with safety margins of 3 mm and it was sent to the Laboratory of Pathology of the same hospital. The specimen was stained with Hematoxylin and Eosin (HE). An immunohistochemical two-step staining technique, using EnVision™+ and anticytokeratin (CK) AE1/AE3, anti-melan A and anti-S100 protein antibodies were also applied on serial sections. The pathological exam revealed a tumor made up of nests of nevomelanocytes, which were located in junctional, intra and subepithelial sites (Figure I.24) (Costea *et al*.,2017c).

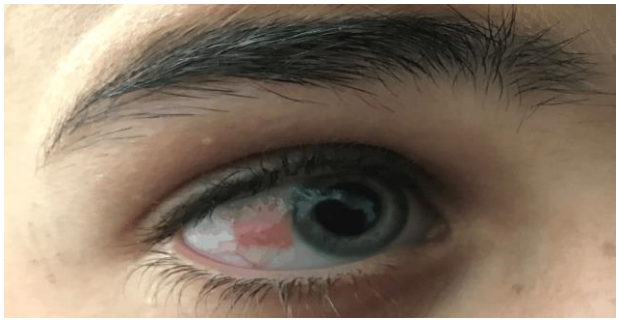

Figure I.23 – Clinical case No. 1. 13-year-old boy with a juxtalimbal, non-pigmented conjunctival lesion with slight irregular borders (gross photography, right eye) (Costea *et al*.,2017c).

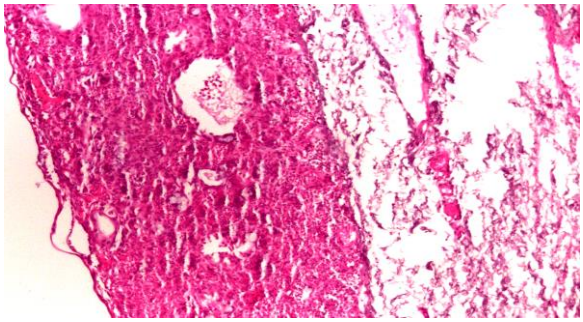

Figure I.24 – Clinical case No. 1. A compound nevomelanocytic proliferation with a nested junctional growth pattern, but also with intra - and subepithelial components. Variable sized epithelial cysts and inflammatory infiltrate could be also seen in the tumoral stroma (HE staining, ×100) (Costea *et al*.,2017c).

Clinical Case No.2

A 12-year-old female patient hospitalized at the same hospital and the same Clinic of ophthalmology, in 2012 complained of a bulbar juxtalimbal, pigmented conjunctival lesion on the left eye with slight irregular borders that had been growing in the last 12 months. The lesion was 4 mm at its widest point, and had a brown coloration with “feeder vessels” (Figure I.25), and was soft in consistency, mobile and painless.

The conjunctival tumor was surgically removed with safety margins of 3 mm and it was sent to the Laboratory of Pathology. The histological sections of the specimen were stained with HE.

The histopathological exam revealed a papillary conjunctival projection due to proliferation of nevomelanocyte cells, which were located at the junction between the epithelium and substantia propria, but also within them both (Figures I.26).

Nevomelanocytes were arranged mainly in nests at the epithelial–subepithelial junction, but there was also noticed a diffuse pattern (Costea *et al*.,2017c).

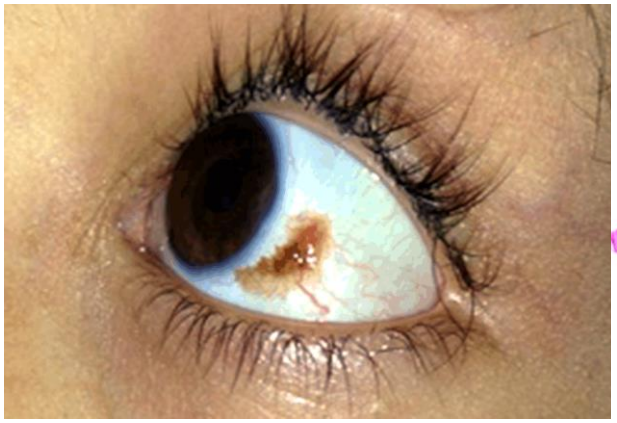

Figure I.25 – Clinical case No. 2. 12-year-old girl with a juxtalimbal, pigmented conjunctival lesion with slight irregular borders and "feeder vessels" (Gross photography, left eye) (Costea *et al*.,2017c).

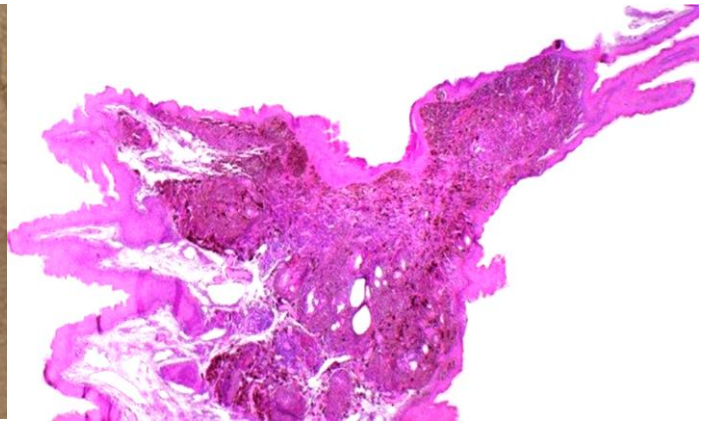

Figure I.26 – Clinical case No. 2. Papillary conjunctival projections due to proliferation of nevomelanocyte cells, which were located at the junction between the epithelium and substantia propria, and within them both (HE staining, ×50) (Costea *et al*.,2017c).

Discussion

Ocular conjunctival nevus occurs clinically in the first and second decades of life, forming from a small nest of cells located at the junction (junctional nevus) and in the second and third decade of life, cells migrate to the stroma (compound nevus) and at this stage intratumoral epithelial cysts get formed. In the third and fourth decades of life, the lesion completely migrates to the conjunctival stroma (subepithelial nevus) (Eslami *et al*.,2013). Clinically, the conjunctival nevus appears as a mobile, hyperemic, well-vascularized lesion located on the bulbar conjunctiva (Barros *et al*., 2009; Thiagalingam *et al*.,2008) and can be confused with inflamed pinguecula, episcleritis, conjunctival cysts, allergic conjunctivitis, squamous epithelial neoplasia, conjunctival sarcoidosis and leukemia (Barros *et al.*, 2009). Although the most common condition is a benign lesion, in children and adolescents it is important to differentiate it from conjunctival melanoma (Shields *et al*.,2004).These benign tumors occur in patients aged 10 to 19 years (Gerner *et al*.,1996) in the bulbar conjunctiva (Novais *et al*.,2010). In the clinical cases studied, the patients were adolescents, a 13-year-old boy and a 12-year-old girl, both lesions being located juxta-bulbar, increasing in size within 12 months. The boy had a non-pigmented nevus, and the girl was diagnosed with a pigmented one (Costea *et al*., 2017c). Shields *et al*., (2004) reported in a study of 410 cases that 65% were brown nevi, 19% were tan, and 16% were non-pigmented nevi (Shield *et al*., 2006). The

clinical aspects of conjunctival nevi can cover a very wide range from intensely pigmented nevi to those with no pigment at all, from very small to large tumors with or without cysts. In the compound nevus, the cystic appearance is most often observed at the clinical examination, which helps the ophthalmologist in differentiating the conjunctival nevus composed of the conjunctival malignant melanoma (Barros *et al*., 2009). Shields *et al*., (2004) found out that all the patients with large nevi over 10 mm had prominent intralesional cysts that could suggest the diagnosis (Shield *et al*., 2004). Both of our cases reported the onset of the disease as a pink or brown conjunctival juxtalimbal lesion that grew in size for approximately one year, being accompanied by inflammation, and causing a sensation of foreign body to the patients (Costea *et al*.,2017c). These two conjunctival lesions did not exceed 10 mm in diameter and probably because the lesions were small, we did not notice any intratumoral cyst on biomicroscopy of the anterior segment of the eye but some epithelial cysts were identified at histopathological exam (Costea *et al*.,2017c). Both of our patients were referred because of a history of moderate "growth" and congestion of the conjunctival lesion for one year. One of the patients had a history of allergic conjunctivitis and this disease was associated with IJCN (Costea *et al*.,2017c). More recently, the American dermatologist Soheil Sam Dadras (2017), described three histopathological characteristics for IJCN as following: unlike adult compound conjunctival nevus, where nevomelanocytes become smaller (nuclear/cytoplasmic ratio decreased) with increasing subepithelial depth, the IJCN shows a paradoxical "reverse" maturation in subepithelium, i.e., nuclear and cytoplasmic size of melanocytes forming subepithelial component is greater than that of junctional component; (Shields *et al.,* 2004) this nevus contains prominent inflammatory infiltrate, which may obscure the architecture of the nevus and can be misleading, giving the impression of cytological atypia (Alkatan *et al*.,2010); this nevus presents intralesional epithelial cysts lined by conjunctival epithelium and goblet cells (Dadras *et al*.,2017). From a histopathological point of view, both of our cases were compound nevi, as they showed a nested junctional growth pattern, along with intra- and subepithelial location, of the nevomelanocytes. Tumoral cells showed different degrees of atypical cytology, but in the second case, it was more obvious. Microscopic examination also revealed epithelial cystic inclusions, and prominent inflammation in the stroma of these two nevi. One of the cases presented heavy inflammation that took the form of lymphoid follicles and eosinophils sheets, but the other showed only diffuse inflammation with lymphocytes, plasma cells, and eosinophils within its stroma (Costea *et al*.,2017c). Immunohistochemical reactions are important for the correct diagnosis of IJCN (Costea *et al.,* 2017c). Even though Jakobiec *et al*., (2010) reported that anti-S100 and anti-melan-A

antibodies were not useful in separating benign from malignant lesions (Jakobiec *et al*.2010), in our case immune staining with anti-S100 and anti-melan-A antibodies was useful for highlighting the tumor cells that were hidden by inflammatory infiltration (Costea *et al*.,2017c). Also, anti-cytokeratin antibody was useful in detecting the tumor cells that were hidden by epithelial cysts (Costea *et al*.,2017c). The conjunctival nevus can progress to conjunctival melanoma in less than 1% of cases (Shields *et al*.,2017; Shields *et al*.,2007). Clinical features that may show malignant transformation of the conjunctival nevus include enlargement of the corneal lesion, adhesion to the sclera, or the presence of 'feeder vessels' (Laver *et al*.,2015). Excision biopsy is not required in the case of small, benign-looking nevi (Albreiki *et al.*,2012), but in the case of large or suspected malignancies, excision is recommended with a safety margin of 3-4 mm (Costea *et al*.,2014), the remaining defect being completed by an amniotic membrane graft or by conjunctivoplasty (Șapte *et al.*,2017). Histopathological examination remains essential in the final diagnosis of these conjunctival hyperpigmentation formations. Especially in young patients, IJCN must be regarded as an independent type of nevus, which might lead even experts in ophthalmic pathology to over-diagnose this lesion as a malignant melanoma (Lommatzsch *et al*.,2007). This event could lead to wrong therapeutic steps with surgical procedures that could cause unnecessary mutilation (Costea *et al.*, 2017c).

Conclusions

IJCN is a conjunctival nevi entity, different from the simple conjunctival compound nevi. They must be differentiated by the pathologist and by the ophthalmologist from the malignant melanoma of the conjunctiva both by clinical, morphological and immunohistochemical evaluation (Costea *et al*., 2017c).

I.2.4. Researches regarding choroidal metastasis and conjunctival malignant melanoma of the eye

Background

Choroidal metastases are rarely mentioned in the literature (Turliuc *et al*., 2015) and this pathology has a frequency of 2% to 9% (Albert *et al*., 1967; Kreuse *et al*., 2022). Choroidal metastasis from lung cancer occurs in the last stage of the disease, and the patient's life expectancy is less than 6 months (Arevalo *et al*., 2005; Asterion *et al.,* 2010). Conjunctival malignant melanoma is a rare tumor that has an increased risk of local recurrence and systemic metastases (Costea *et al*., 2014). This tumor has an incidence of 1.6% of all non-cutaneous melanomas (Shields *et al.*,2000) and 5% of all ocular melanomas (Isager *et al*.,2002). Conjunctival melanoma is more common in adults and elderly, the majority of

patients being older than 40 years. Invasive conjunctival melanoma can be heavily pigmented, sparsely pigmented, or amelanotic. Tumors may be unifocal or multifocal (Damato *et al.*,2009). The patients with advanced conjunctival melanoma identified clinically and confirmed histologically have a poor prognosis due to the development of lymph node and systemic metastases (Brownstein, 2004; Seregard,1998; Jacobiec *et al.*,1988). Conjunctival melanoma invades locally the subepithelial region and metastasizes at distance to the regional lymph nodes (Damato *et al.*,2009).

These metastases occur because the subepithelial conjunctival stroma contains blood and lymphatic vessels (Brownstein, 2004).

A rare case of choroidal metastasis that was diagnosed in the Second Clinic of Ophthalmology and treated for brain metastasis in the Second Clinic of Neurosurgery of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iași, Romania and the case was published in a journal indexed in the ISI Web of Science and set out. I will also present below a rare case of conjunctival malignant melanoma, which was diagnosed and treated in the Second Ophthalmology Clinic of the “Prof.Dr.Nicolae Oblu”Emergency Clinical Hospital Iași, Romania. The case was published in an ISI Web of Science indexed journal with an impact factor, also set out below.

Turliuc MD, Sava A, Dumitrescu GF, Cucu A, Esanu A, Tudorache C, Costache II, **Costea CF**. Right visual loss due to choroidal metastasis of a papillary adenocarcinoma of the lung: a case report, *Romanian Journal of Morphology and Embryology*, 2015, 56(3): 1173-1177. IF=0.811.

Costea CF, Anghel K, Dimitriu G, Dumitrescu GF, Faiyad Z, Dumitrescu AM, Sava A. Anatomoclinical aspects of conjunctival malignant metastatic melanoma, *Romanian Journal of Morphology and Embryology*, 2014, 55(3): 933-937. IF=0.659

Clinical Case of a Choroidal Metastasis

In 2015, we reported the case of a 40-year-old patient, chronic smoker, who was hospitalized in the Department of Neurosurgery of the "Prof. Dr. Nicola Oblu"Emergency Clinical Hospital Iași, Romania for headache, right motor deficit and visual loss in the right eye. Chest CT scan highlighted a tumor located in the lower pulmonary lobe, and the cytopathology examination of bronchial brushing specimens performed at the Hospital for Lung Diseases a week previously had revealed a non-small cell lung carcinoma (Figure I.27).

The ophthalmological exam revealed no light perception of the right eye with serous retinal detachment.

Moreover, slit lamp exam of the right eye showed a greyish aspect of the pupil (Figure I.28).

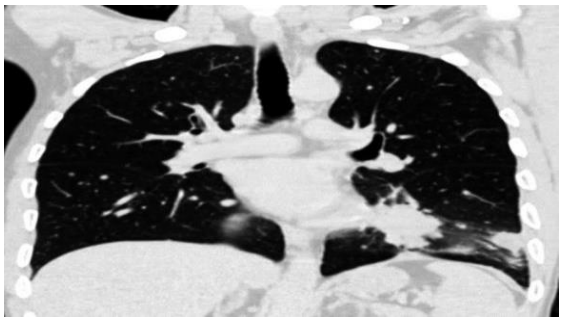

Figure I.27 - Chest contrast CT scan showing the tumor in the left lung (Turliuc *et al.*, 2015).

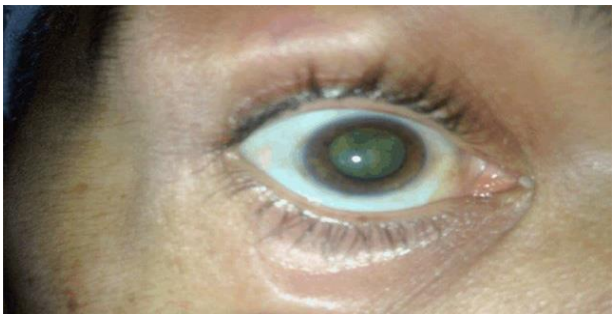

Figure I.28 - Right eye: greyish appearance of the pupil (serous retinal detachment) (Turliuc *et al*., 2015).

Head CT scan with contrast highlighted multiple supratentorial metastasis in the left hemisphere, one of which was larger, located in the left rolandic region (Figure I.29), and also another lesion in the right eyeball posterior pole (Figure I.30).

After surgical resection of the left rolandic lesion, the tissue obtained was processed by the usual histopathological technique and the final pathological exam revealed the diagnosis of brain metastasis of a bronchopulmonary papillary adenocarcinoma (Figure I.31) (Turliuc *et al*.,2015).

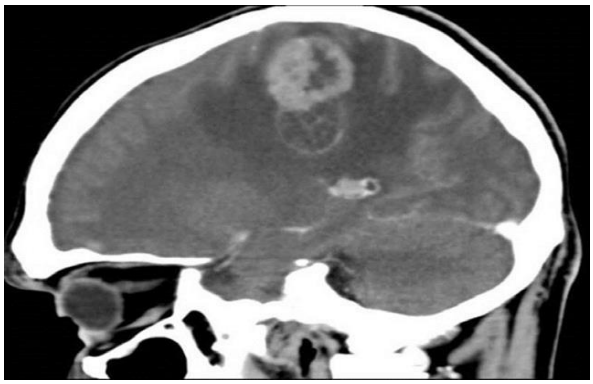

Figure I.29 - Head CT scan with contrast showing the parenchymal metastasis (Turliuc *et al*., 2015).

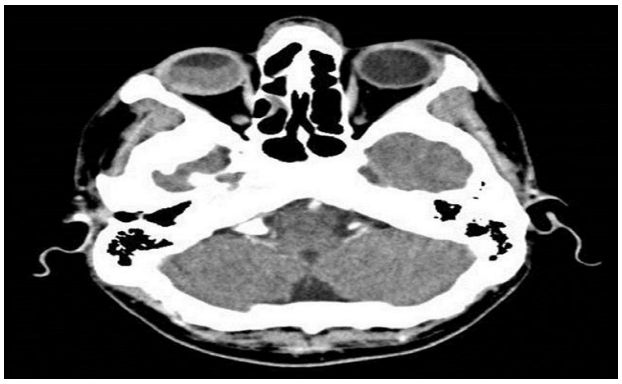

Figure I.30 - Axial contrast CT scan showing intense enhancement in right eye in close relation to the choroid (Turliuc *et al.*, 2015).

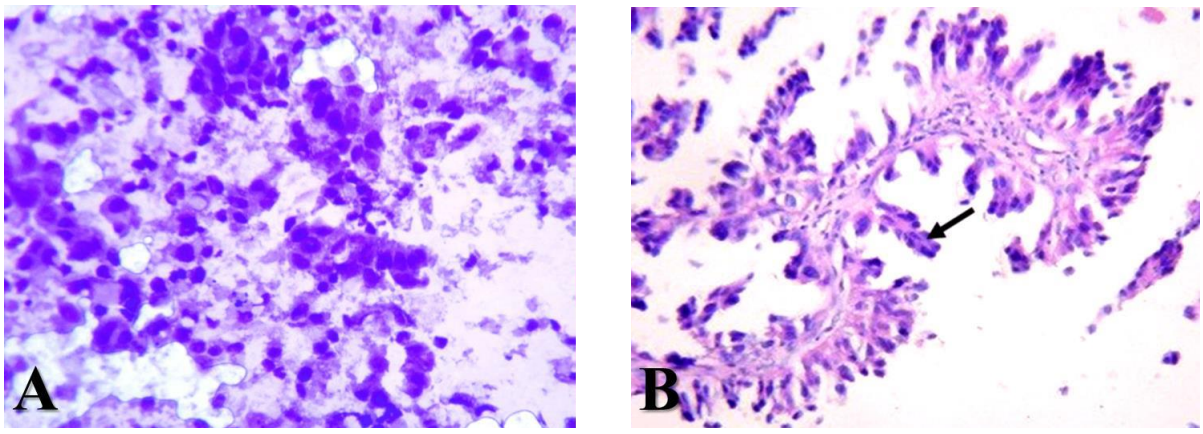

Figure I.31. An intraoperative imprint taken from left rolandic tumor revealed nests of malignant epithelial cells (toluidine blue staining, x400) (A). High-power photomicrograph of left rolandic lesion: micropapillary features lacking fibrovascular core (black arrow) (HE staining, x400) (B) (Turliuc *et al*., 2015)

Discussions

In the Romanian medical literature, cases of choroidal metastasis are extremely rare, being published only 3 articles so far (Fumarel *et al*., 2008; Munteanu *et al*., 2013; Munteanu *et al*., 1994). In the case of our patient, the CT scan showed multiple supratentorial metastases in the left hemisphere, one of which was large and located in the left rolandic region, which was surgically resected (Turliuc *et al.*, 2015).

Imaging diagnosis shows that 22% of cases of eye metastases are combined with brain metastases (Mewis and Young, 1982).

The presence of choroidal metastasis is a sign of unfavorable prognosis, indicating the final stage of lung cancer (Aragao *et al*., 2013; Asteriou *et al.*, 2010). The treatment of these intraocular metastases is palliative and consists of orbital exenteration, enucleation, chemotherapy and orbital irradiation (Asterion *et al.*, 2010; Dobrowsky, 1988).

Conclusions

Choroidal metastasis from bronchopulmonary cancer is a rare pathology in our country. Diagnosis and treatment of patients diagnosed with bronchopulmonary cancer with brain and eye metastases involves interdisciplinary collaboration. Ophthalmic screening of all patients with brain metastases is of paramount importance (Turliuc *et al*.,2o15).

Clinical Case of a Conjunctival Malignant Metastatic Melanoma

In 2012, a 33-year-old male patient was admitted to the Second Ophthalmology Clinic of the "Prof.Dr.Nicolae Oblu"Emergency Clinical Hospital Iași, Romania, with two tumors located on the left upper palpebral conjunctiva. History revealed that the patient was clinically

diagnosed also with left bulbar conjunctival melanosis. One month before presentation, the patient noticed a left upper eyelid prominence, foreign body sensation, and bloody discharge. The clinical diagnosis was nodular melanoma of the left upper eyelid conjunctiva and bulbar conjunctival melanosis.

The tumors were surgically removed with safety margins and topical 5-Fluorouracil treatment was applied. Tumor excision was followed by conjunctivoplasty using lower lip mucosa. For one year, the patient refused referral to a specialized service.

In 2013, the patient was admitted in emergency to the 3rd Neurosurgery Unit in the same hospital with first grade coma, right hemiplegia, aphasia, and jaundice syndrome.

CT scan of the brain identified a left parietal tumor, which was surgically excised. Subsequently, while admitted to the Oncology Clinic, multiple cutaneous, lung, liver, peritoneal, renal metastases and peripancreatic lymph node metastases were detected. The patient died three months after the surgical excision of brain metastasis. Tumor fragments collected from the conjunctival tumors and brain metastasis were fixed in 4% formalin, embedded in paraffin, and stained with Hematoxylin–Eosin (HE). For immunohistochemical reactions, were used three monoclonal antibodies (anti-HMB45, anti-S100 protein, anti-vimentin) (Costea *et al*.,2014).

Results

The ophthalmologic examination consisted of an average ptosis caused by two vegetative, ulcerated formations located on the tarsal conjunctiva of the left upper eyelid (Figure I.32) (Costea *et al*.,2014).

Biomicroscopic examination revealed areas of pigmentation in the bulbar conjunctiva in the external paralimbic sector in the left eye suggesting conjunctival melanosis. Local examination did not show any periauricular and submandibular lymphadenopathy.

Excision of the conjunctival formation from the left upper eyelid was performed and histopathological examination established the diagnosis of invasive amelanotic malignant melanoma of the conjunctiva, subtype epithelioid, *de novo* developed, histological grade G3 (Figure I.33) (Costea *et al*., 2014).

The tumor consisted of large, irregular tumor cells with abundant eosinophilic cytoplasm, atypical nuclei, and numerous atypical mitoses (three mitoses/medium power field) (Figure I.33).

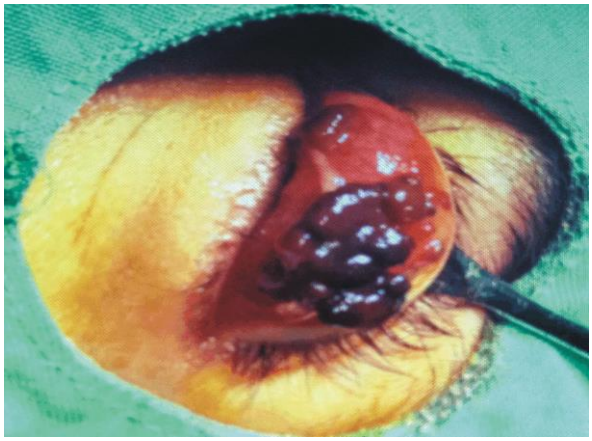

Figure I.32 – Conjunctival malignant melanoma of the left upper eyelid (Costea *et al*.,2014).

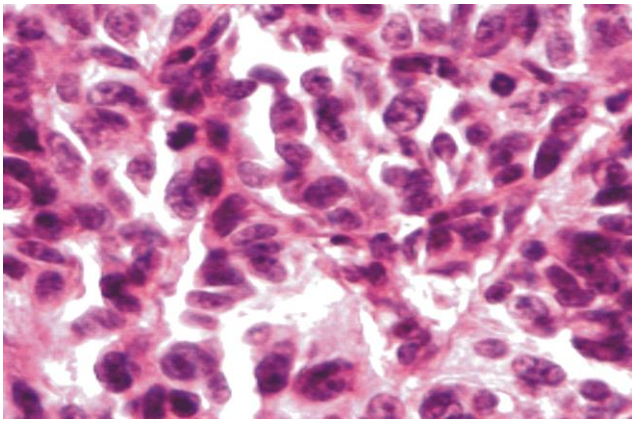

Figure I.33 – Amelanotic, epithelioid conjunctival malignant melanoma. Sheets of epithelioid neoplastic melanocytes with marked atypia and numerous atypical mitoses. The tumor sheets are surrounded by connective-vascular tissue septa (HE staining, ×400) (Costea *et al*.,2014).

In tumor tissue, binucleated and multinucleated giant neoplastic melanocytes were also identified. Tumor stroma (brain metastasis) was composed of connective tissue septa with capillary vessels (Figure I.34).

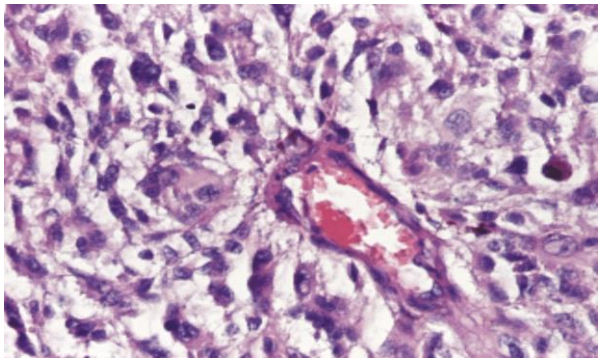

Figure I.34 – Brain metastasis from amelanotic, epithelioid conjunctival malignant melanoma. Tumor tissue is composed of multinucleated epithelioid and giant neoplastic melanocytes, with abundant eosinophilic cytoplasm (HE staining, ×200) (Costea *et al*.,2014).

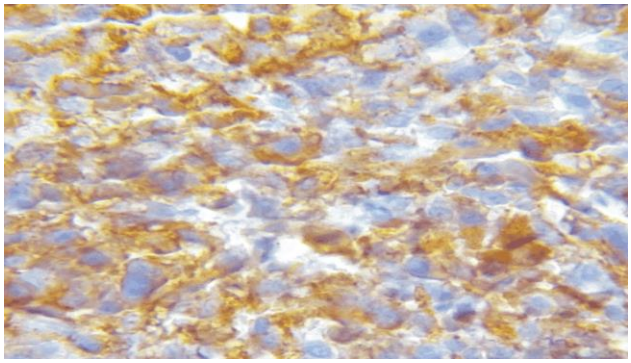

Figure I.35 – Amelanotic, epithelioid conjunctival malignant melanoma. Cytoplasm of epithelioid neoplastic melanocytes is highly positive for HMB45 (IHC staining, ×100) (Costea *et al*.,2014).

Immunohistochemical reactions were positive for HMB45, S100 protein, and vimentin in the neoplastic melanocytes of conjunctival malignant melanoma and brain metastasis (Figures I.35–I.37) (Costea *et al*.,2014).

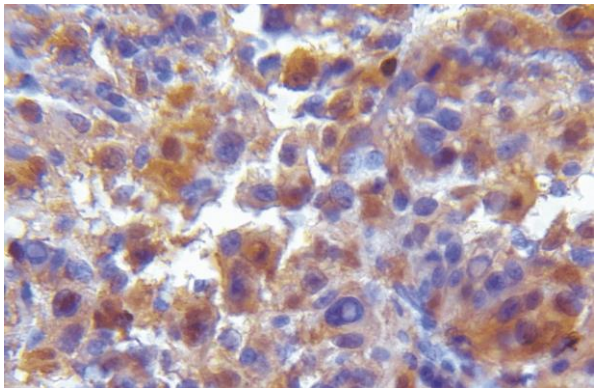

Figure I.36 – Amelanotic, epithelioid conjunctival malignant melanoma. Cytoplasm of epithelioid neoplastic melanocytes is highly positive for S100 protein (IHC staining, ×200) (Costea *et al*.,2014).

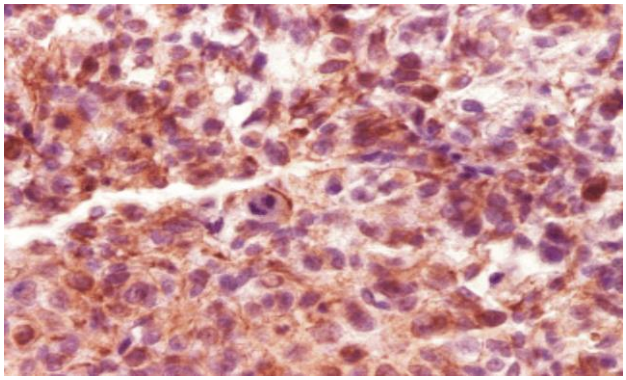

Figure I.37 – Amelanotic, epithelioid conjunctival malignant melanoma. Cytoplasm of epithelioid neoplastic melanocytes is highly positive for vimentin (IHC staining, ×40) (Costea *et al*.,2014).

Discussions

Conjunctival malignant melanomas arise from melanocytes located in the basal layer of conjunctival epithelium, cells derived from the neural crest (Jovanovic *et al.*,2013). Conjunctival melanoma has an increased potential for metastasis and an increased mortality and morbidity rate (Shields *et al.*,2011; Esmaeli *et al*.,2012; Sgildkrot *et al.*,2010). Conjunctival malignant melanoma metastasizes initially in the preauricular and submandibular lymph nodes (Shields *et al*.,2004; Holland *et al.*,2013) and then by contingency in the nasal cavity (Choi *et al*.,2005), eyeball, orbit and paranasal sinuses (Gökmen *et al*.,2008). Distant blood metastases occur in the lung (Missotten *et al*.,2005), liver, digestive tract (Cohen *et al*.,2007), brain (Shields *et al*.,2000), bones (Manidakis *et al*.,2009), parotid glands (Cothbertson *et al*.,2003) and skin (Hollan *et al*.,2013; Missotten *et al*.,2005). Median times to regional and systemic metastases are approximately 2.3 and 3.4 years, respectively (Tuomaala *et al*.,2004).

Our patient was diagnosed with brain, lung, liver, kidney, peritoneal, subcutaneous, and peripancreatic lymph node metastases at 1.9 years after primary tumor removal (Costea *et al*.,2014). Incomplete tumor excision was demonstrated by many studies as a poor prognostic factor (Brownstein, 2004). Paridaens *et al.*, (1994), in a study of 256 patients found that conjunctival malignant melanoma has an unfavorable prognosis when located in the

conjunctival fornix, caruncle and eyelid margin, tumor thickness greater than 4 mm, multicenter appearance, epithelioid cells and lymphatic invasion, the mortality rate being 2-5 times higher (Paridaens *et al*.,1994).

Our patient was in the young age group, and the conjunctival melanoma was located in the non-bulbar conjunctiva, was multicentric, with superficial ulcerations, deep tumor invasion (8 mm), it was composed of epithelioid tumor cells with numerous mitoses (average 20/10 highpower fields) (Costea *et al*., 2014). The conjunctival melanoma was classified as stage pT4b, pN3, pM1 according to AJCC classification for conjunctival melanoma (Compton *et al*.,2012). Depending on the clinical study, the treatment of patients with conjunctival malignant melanoma requires various treatment methods: surgical excision in 3-4 mm safety margins, brachytherapy, cryotherapy and in some cases orbit exenteration (Shields *et al*.,2000; Brownstein,2004; Jovanovic *et al.*,2013; Saornil *et al*.,2009).

Conclusions

Conjunctival malignant melanoma is a rare tumor. Unfavorable prognostic factors are tumor thickness over 2 mm, tumor ulceration, increased mitosis rate and epithelioid cell type. Patients diagnosed in advanced stages of the disease have an unfavorable prognosis, the tumor often cannot be completely excised (Costea *et al*., 2014).

I.2.5. Researches regarding orbital and sino-nasal tumors in adults

Background

Orbital tumors are a common topic today, due to the complexity of clinical and imaging diagnosis, but also complex and challenging therapeutic management (Dorssant *et al*., 2021).

Shields *et al.* published a study in 2014 on 1264 patients who were diagnosed with an orbital tumor or other condition that mimicked a tumor and found that 17% of them were vascular lesions, followed by lymphoid tumors, lacrimal gland tumors, optic nerve and meningeal tumors metastasis, peripheral nerves and primary melanoma lesions (Shields *et al*., 2004; Tailor *et al.*, 2013).

The approach to orbital and sinonasal tumors often requires interdisciplinary collaboration, the ophthalmologist, neurosurgeon, maxillofacial surgeon and oncologist having an important role in the complex management of these cases. Most of these tumors have the same ophthalmological signs: exophthalmos and diplopia.

I published several articles on orbital and sino-nasal tumoral pathology, one of which is in an ISI Web of Science indexed journal. These articles are presented below.

Costan VV, Sava A, Carauleanu A, **Costea CF**, Cucu AI, Dimitriu G, Dumitrescu GF, Dumitrescu N, Stoicescu MS, Raftu G, Turliuc MD. Histopathological and Clinical Characteristics of Surgically Removed Cavernous Venous Malformations (so-called Cavernous Hemangiomas) of the Orbit, *Revista de Chimie*, 2019, 70(1): 350-354. IF=1.605.

Costea CF, Turliuc D, Costan VV, Faiyad Z, Dumitrescu GF, Cucu A, Sava A. Unilateral exophthalmos in a case of maxillary sinus carcinoma with orbital invasion, *Revista Romana de Anatomie Functionala si Clinica, Macro- si Microscopica si de Antropologie*, 2015, 14(3): 457-461.

Turliuc D, Trandafir D, Cucu A, Dobrin N, Dumitrescu G, Sava A, Dumitrescu AM, **Costea CF.** Giant nasopharyngeal carcinoma – a case report dynamic anatomical models in skull base and intracranial space invasion, *Romanian Journal of Oral Rehabilitation*, 2016c, 8(1): 51-58.

I.2.5.1. Histopathological and clinical characteristics of surgically removed cavernous venous malformations of the orbit

In a retrospective study conducted over 7 years (2010-2017) we analyzed 14 patients diagnosed with orbital cavernous hemangiomas.

Patients' sociodemographic, clinical, radiological, and surgical findings were retrospectively retrieved from medical records and analyzed. Diagnosis of cavernous venous malformations (CVM) was confirmed histologically in all cases. For this study, the pathologists reviewed all histological samples. The ophthalmic examinations included visual acuity (VA) measurement with Snellen charts, motility of eyes, pupillary reflexes, proptosis measurement with Hertel exophthalmometer, Goldmann tonometry for intraocular pressure, and fundus examination with direct ophthalmoscope.

The indications for surgical therapy included the presence of at least one of the following signs and symptoms: visual impairment, progressive and disfiguring unilateral proptosis, double vision and pain. Preoperatively, 6 patients underwent MRI, 5 underwent CT and the rest of them underwent both investigations without contrast. Tumor excision was performed by transconjunctival approach or transcutaneous incision. The resected specimens were examined histopathologically and stained with Hematoxylin and Eosin (H and E). In all cases, the diagnosis of CVM was recorded. All patients were followed postoperatively for at least 6 months (Costan *et al.*,2019).

Results and discussions

All patients were women and their ages varied from 13 to 57 years (mean age was 44.2 years ± 5.7 years). In this study, the main presenting sign among these patients was proptosis in ten cases (71.42%) (Figure I.38), but seven patients (50%) presented diplopia, too. In four cases (28.57%), the lesion was discovered incidentally at imaging evaluations for persistent headache. The left orbit was affected in eleven cases (78.57%) and the right orbit in three cases (21.42%). No case was bilaterally involved. CT images of patients included in the study revealed a round well-defined soft tissue tumor (Figure I.39).

At MRI the aspect of the tumor was similar to CT images, but the signal in T1 was isointense compared to the muscle, and in T2 hyperintense compared to the muscle. Our cases were located in the extraconal inferior or medial orbital compartments, so the surgical treatment was represented by anterior orbitotomy. In twelve cases (85.71%) we performed tumor excision using the transconjunctival approach and in two cases (14.28%) we chose the transcutaneous incision.

Figure I.38 - Patient with displacement of the left eye opposite to the position of the tumor (Costan *et al.*,2019).

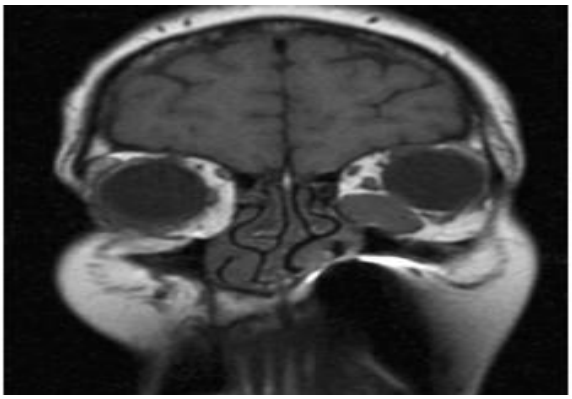

Figure I.39 - Axial CT showing a well defined ovoid lesion (Costan *et al.*,2019).

Total tumor resection was achieved in all the cases (Figure I.40) (Costan *et al.*,2019). Histopathologically, the tumors had a fibrous pseudo-capsule that encapsulated each of the resected tumors (Figure I.41, A and B). At six months postoperatively, the proptosis partially recovered and visual acuity improved, with no recurrences (Costan *et al*.,2019). The name cavernous hemangioma has in recent years been replaced in the medical nomenclature with venous cavernous malformations (CVM) (Rootman *et al.*,2014). These tumors are common, rarely in children and frequently in adults aged 40 to 60 years, mostly female (60-70%) (Rootman *et al.*,2014; Ansari and Mafee, 2005).Our cases were women with an average age

between 40 and 60 years (Costan *et al.*,2019), comparable to other studies (17-19). (Harris and Jakobiec,1979; McNab and Wright,1989; Savoiardo *et al*.,1983).

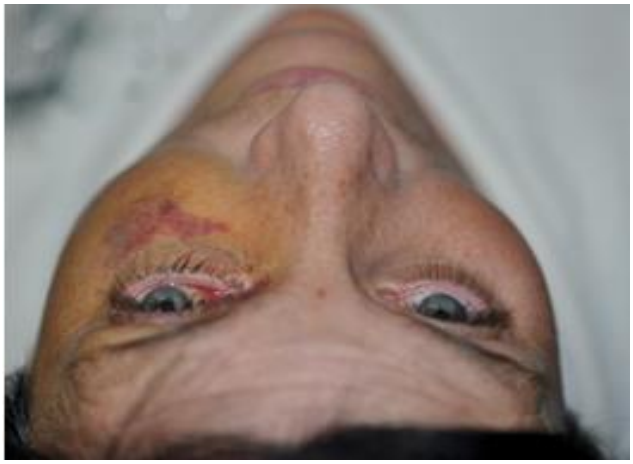

Figure I.40 - Postoperative evolution following transcutaneous incision, 7 days after surgery (Costan *et al.*,2019).

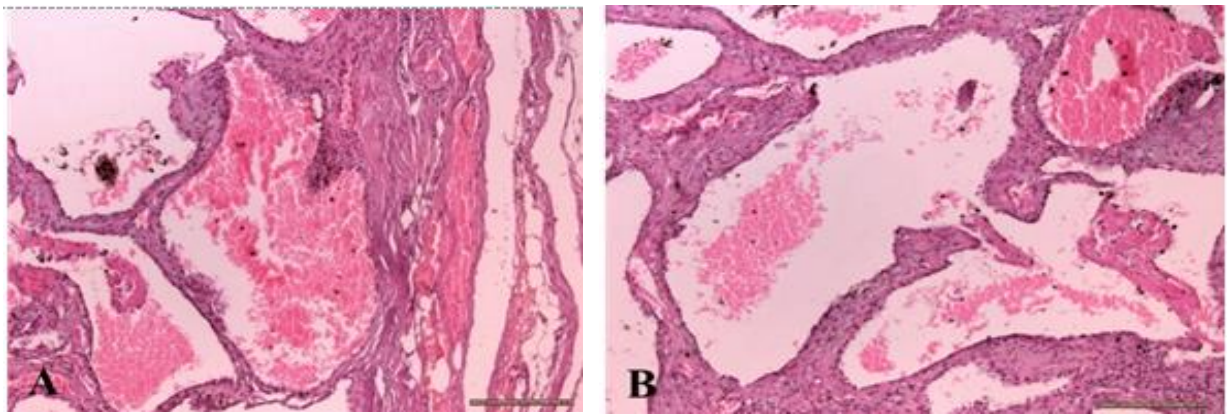

Figure I.41 - Histopathological images of orbital cavernous venous malformation: Case 1. A). Large cavernous vascular channels filled with blood and lined by a single layer of flattened endothelial cells and separated by scant connective tissue stroma. A fibrous pseudocapsule surrounded the lesion (H and E staining, x10) B). The central part of the lesion showed large, endothelium-lined, blood-filled spaces, which are tightly knit and separated by thick septa (H and E staining, x10) (Costan *et al*., 2019).

The most frequent location is the retrobulbar compartment of the orbit, especially in the lateral part (Ansari and Mafee, 2005; McNabb *et al*.,1990). The most common sign and symptom is proptosis, which is present in 70-95% of cases (Scheuerle *et al.*,2004; Calandriello *et al*.,2017; Hsu and Hsu,2011). Another common symptom is impairment of visual acuity (50%), which appears when the hemangioma compresses the optic nerve. Also, local pain, visual field defects, diplopia, and chronic headache can appear in less than 20% of cases (Scheuerle *et al.*,2004). Similar to other studies, we encountered proptosis in almost

three quarters of our cases, all females, but we found a higher rate of diplopia, probably due to the small sample size of the tumor (Costan *et al.*,2019).

A palpable mass is rarely present in cases with cavernous hemangiomas and optic atrophy is also noted occasionally. When the tumor is located in the vicinity of the globe, it may induce hyperopia and choroidal folds, which interestingly, persist even after complete removal of the hemangioma (Gunduz and Karcioglu,2015). Histopathological examination of a cavernous hemangioma reveals a benign vascular malformation characterized by a well-defined capsule and numerous large dilated vascular channels lined by endothelial cells with an intervening fibrous interstitium (Ansari and Mafee,2005; Calandriello *et al*.,2017; Smoker *et al.*,2008). Imaging examination (CT, MRI) reveals a well-circumcised round tumor mass or an orbital ovoid mass (Khan and Sepahdari,2012; Yan and Li, 2014). In our cases, we identified a mixture of venous muscular channels and of capillary structures (Costan *et al*.,2019).

All these structures were separated by thick strands of connective tissue, as other authors reported (Osaki, *et al.*,2013).

We identified in the walls of large vascular channels, variable multilaminar smooth muscle-like bundles under the endothelium, the image being most closely to dysplastic veins. So, we concluded that the so-called orbital cavernous hemangioma is rather a cavernous venous malformation (Costan *et al*.,2019). Anterior orbitotomy is the operation of choice for extraconal and intraconal tumors, which do not involve the orbital apex (Smoker *et al*.,2008).

For small tumors located near the eyeball, the transconjunctival approach can be used (Calandriello *et al*.,2017; Nagasaka *et al*.,2007). Postoperative complications of the previous approach are eyelid hematoma, mydriasis, loss of visual acuity when the optic nerve is compressed, arterial occlusion, entropion or ectropion (Rootman *et al*.,2014). In the case of our patients no complications were observed, the postoperative results were favorable in terms of proptosis and diplopia (Costan *et al*., 2019).

Conclusions

Our series of cases demonstrated that orbital cavernous hemangiomas express clinical and imaging features of a benign lesion which occurs usually in adult females. Surgery was recommended in the presence of clinical manifestations (proptosis and diplopia).

Having to deal with extraconal lesions, we successfully used anterior orbitotomy, mainly the transconjuctival approach, with no complications or recurrences after a six-month follow-up period (Costan *et al.*,2019).

I.2.5.2. Studies on malignant sino-nasal tumors

Background

Malignant tumors of the nasal cavity and paranasal sinuses are rare and represent 1% of all malignant tumors, the most common being poorly differential squamous cell carcinoma of the maxillary sinus (Osguthorpe *et al.*,1979; Bhattacharyya,2003; Jham *et al*.,2006). Nasopharyngeal carcinoma (NC) develops in the lateral walls of the nasopharynx and spreads to the top, bottom, to the anterior and posterior sides (Li *et al.*,2013).

The dimensions of the malignant tumors of the maxillary sinus can considerably increase before the visit to a physician and they require an aggressive therapy, taking into account the location close to the base of the cranium, orbit, and cranial nerves.

Although rare, they can be of great importance, producing in the initial stages symptoms and clinical signs which mimic common inflammatory diseases.

That is why the patient and the physician often ignore or minimize these tumors during the initial consultations, treating them as inflammatory or benign diseases of the nasal sinuses region (Jham *et al*.,2006; Klem,2015).

The maxillary sinus carcinoma frequently affects men in the sixth or seventh decade of life (Jham *et al.*,2006; Waldron *et al.*, 2000).

Clinical Case No.1

We presented the case of a 46-year-old female patient hospitalized in the second Ophthalmology Clinic of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania, for left unilateral exophthalmos, epiphora and hemicrania.

The patient's medical history revealed numerous dental extractions and chronic treatments for headaches, recommended by the family doctor, being treated with antibiotics and non-steroidal anti-inflammatory drugs for 12 months.

The local and general symptoms did not heal with the treatments, but worsened, the patient presenting urgently to the ophthalmologist.

Ophthalmologic examination revealed a non-reducible unilateral exophthalmos with external vertical deviation of the eyeball, diffuse non-inflammatory edema of the left upper eyelid, visual acuity was normal in both eyes, eye pressure as well.

Biomicroscopic examination revealed conjunctival chemosis in the left eye (Figure I.42) (Costea *et al*., 2015).

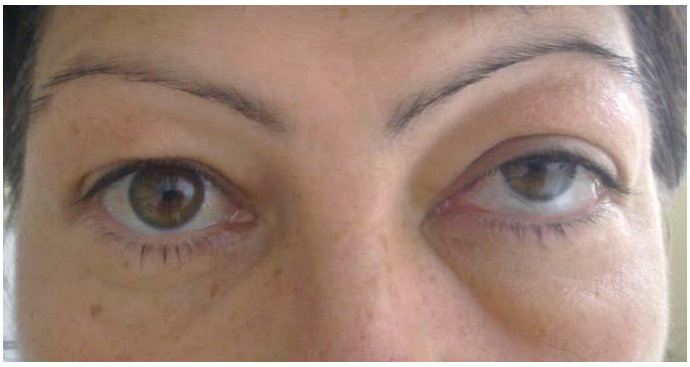

Figure I.42. Unilateral exophthalmos with vertical deviation upward of the left eyeball (Costea *et al*.,2015).

A discrete backward displacement of the left eyeball was identified. The optic nerve and the other ocular motor muscles had normal tomodensitometry (TDM) appearance. There were no suggestive elements for an eventual intracerebral extension (Costea *et al*.,2015).

Examination of the craniocerebral and orbital CT showed a parenchymal tumor formation with homogeneous structure located at the level of the left maxillary sinus, which produced the destruction of its superior and internal walls, and the small wing of the sphenoid.

It was noticed the extension of the tumor mass into the nasal cavity, the ethmoidal cells, zygomatic lodge and left intraorbital region, incorporating the right inferior muscle (Figures I.43 and I.44).

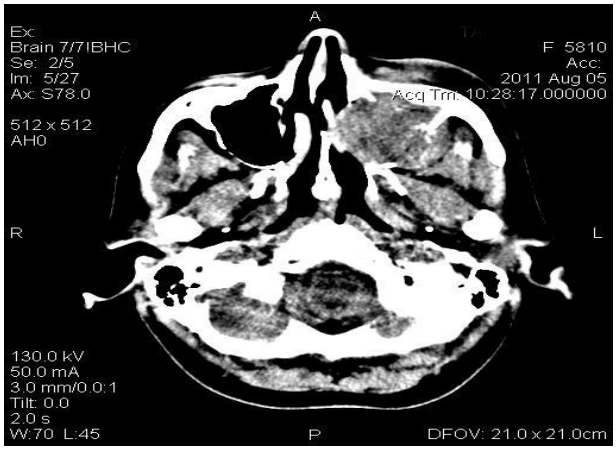

Fig. I.43. Axial CT image shows a homogenous tumoral mass situated into the left maxillary sinus that invaded the nasal cavity (Costea *et al*.,2015).

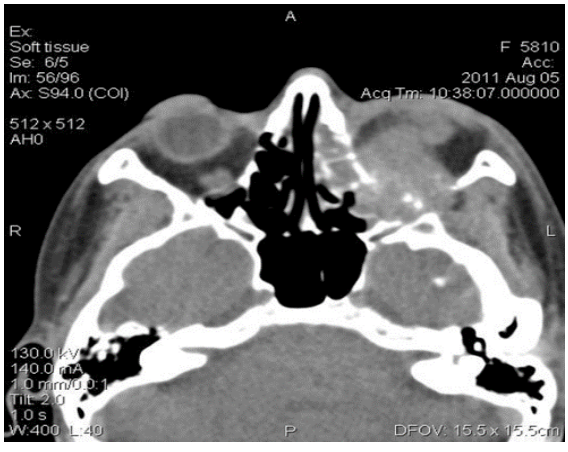

Fig. I.44. Axial CT image shows a homogenous mass with starting point at the level of the left maxillary sinus. The tumoral formation invades the left orbit and ethmoidal cells (Costea *et al*.,2015).

Results and discussions

The surgery was performed in the Neurosurgery Clinic, of the Emergency Clinical Hospital "Prof.Dr.Nicolae Oblu" Iași, Romania, partial tumor resection and also exanteration also orbit (Costea *et al*., 2015). The established histopathological diagnosis was poorly differentiated carcinoma with starting point in the mucosa of the maxillary sinus (Figure I.45).

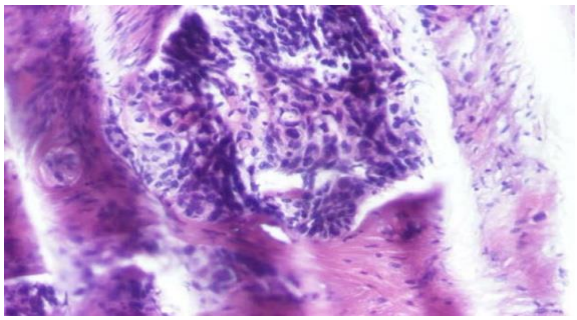

Fig. I.45. Microphotograph of the excised specimen: maxillary sinus poorly differentiated squamous cell carcinoma, basaloid variant showing an island of poorly differentiated epithelial cells with eosinophilic cytoplasm, atypical nuclei and some intercellular bridges. The peripheral tumor cells are disposed in a palisade shape (Hematoxylin-Eosin coloration, x 200) (Costea *et al*.,2015).

The patient was referred to the Oncology Clinic where she was treated with chemotherapy and palliative radiotherapy, dying 6 months later. Most orbital neoplasms originate in the paranasal sinuses. More than 50% of patients with sinus tumors may show oculo-orbital clinical signs (Johnson *et al*.,1984; Myers and Oxford, 2004) . The mucosa of the maxillary sinus cavity is the frequent origin of the secondary orbital tumors, the most common being the squamous cell carcinoma. The maxillary sinus carcinoma is more frequently in males (male/female= 2.3/1) (Jham *et al*.,2006). Its extension modality into the orbit is through bone erosion or through bone channels, the inferior orbital wall being extremely thin (Jakobiec's,2008). The maxillary sinus squamous cell carcinoma invades the pterygoid region and the patient often comes to the dentist with toothaches (Esposito *et al*.,2006; De Monte *et al*.,2000) in our case, the patient had toothaches and underwent tooth extractions, this being the first sign of the carcinoma invasion (Costea *et al*.,2015). The literature highlights the following clinical signs of the maxillary sinus tumors with orbital invasion (Jakobiec's,2008): exophthalmos, congestion and conjunctival chemosis, strabismus, visual acuity decrease, nasal tumor mass. As for our patient, the orbital invasion produced ocular clinical signs, determining the unilateral nonaxial nonreducible exophthalmos and the vertical upward displacement of the left eyeball, with conjunctival chemosis (Costea *et al*.,2015). The survival of patients diagnosed with maxillary sinus carcinoma with invasion of the orbit is 25-35% at 5 years (Jakobiec's,2008). The prognosis is extremely unfavorable for

poorly differentiated squamous cell carcinoma of the maxillary sinus with orbital invasion and cranial invasion (Jham *et al*.,2006; Jakobiec's, 2008).

Conclusions

Exophthalmos may be a sign of orbital invasive maxillary sinus carcinoma. This tumor is extremely aggressive and can mimic other diseases, which delays the correct and early diagnosis, the patient's prognosis becoming poor. In order to improve the survival of patients diagnosed with maxillary squamous sinus carcinoma with orbital invasion, management should involve a multidisciplinary approach as the areas involved are complex (Costea *et al*.,2015).

Clinical case No.2

A patient, a 60-year-old man, came urgently to "Prof. Dr. Nicola Oblu" Emergency Clinical Hospital Iași, Romania, with headache and diplopia. CT examination revealed a giant tumor located in the nasopharynx extending into the nasal cavities, ethmoid cells, sphenoid sinus in the selar, suprasellar intracranial space, cavernous sinuses, carotid canal, and prepontine cistern (Figures I.46, A and B).

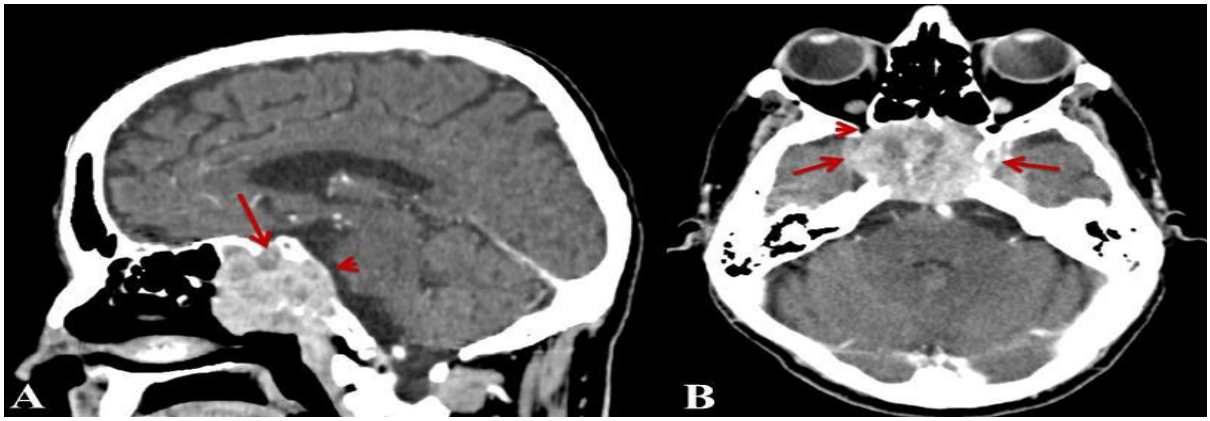

Figure I.46 A. Head contrast-enhanced CT (sagittal image) where sella turcica (arrow) and clivus (arrowhead) are observed; B. Axial contrast-enhanced CT image where the right orbital apex invasion (arrowhead) and bilateral cavernous sinus invasion(arrows) are observed (Turliuc *et al*.,2016c).

Tumor biopsy revealed a nasopharyngeal carcinoma. Histopathological examination of Hematoxizin-Eosin (HE) stained specimens revealed epithelial cells disposed in a syncytial pattern of growth and included in a fibrous background infiltrated with lymphocytes and plasmocytes (Figure I.47).

The positivity of tumor cells for pancytokeratin established the final diagnosis of a nasopharyngeal nonkeratinizing carcinoma, a non-differentiated subtype (Figure I.48) (Turliuc *et al*.,2016c).

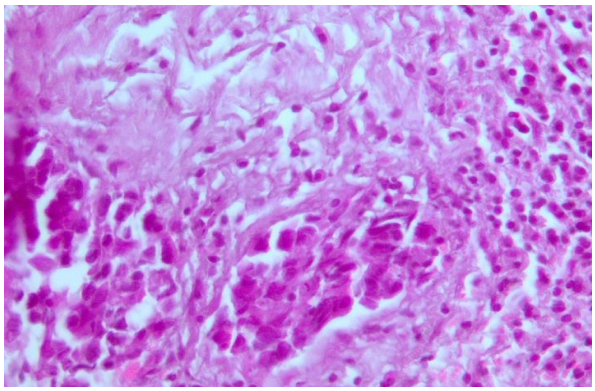

Figure I.47. Histopathological exam of the biopsy: the tumour comprises irregular islands of neoplastic undifferentiated epithelial cells surrounded by a desmoplastic stroma infiltrated with mononuclear inflammatory cells. The syncytial-appearing large tumour cells have indistinct cell borders, and a scant eosinophilic cytoplasm (Hematoxylin and Eosin staining, x 400) (Turliuc *et al*.,2016c).

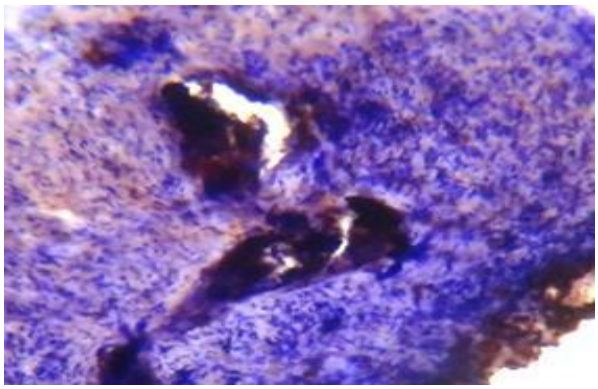

Figure I.48. Immunostaining for pan-cytokeratin highlights the irregular clusters of strongly positive tumor cells located in the stroma that is infiltrated with unstained mononucleated inflammatory cells (AE1/AE3, Dako; x 200) (Turliuc *et al*.,2016c).

Discussions

NC is a distinct type of cancer that differs from other malignancies of the upper respiratory and digestive tracts in epidemiology, etiology, pathology, clinical appearance, and treatment (Agulnik and Epstein, 2018; Mackie *et al*.,2000; Chen *et al*.,1989). The symptoms are often poor and are related to the involvement of the temporo-mandibular joint (Agulnik and Epstein, 2018). Exophthalmos may occur if the orbit is invaded (Turliuc *et al.*,2016c). The nasopharyngeal anatomy is located below the skull base and bounded, as it shows in Figure I.49. Invasion of the skull base occurs in 25-65% of cases with NC (King *et al*.,1999; Roh *et al*.,2004) and is one of the unfavorable prognostic factors (Li *et al.*,2013; Lu *et al*.,2001; Liu *et al*.,2009), with an increased risk of metastasis (Li *et al*.,2013). Invasion of the prepontine cistern (Figure I.50) it is also a rather reserved prognosis (Hung *et al*.,2014). The internal surface of the skull base is divided into three regions: anterior cranial fossa, middle cranial fossa and posterior cranial fossa crossed by numerous foramina and fissures through which the NC could expand to the intracranial space. Out of these, the most frequently invaded in NC is the middle cranial due to its proximity to the nasopharynx (Figure I.51,A) (Turliuc *et al.*,2016c). Some studies have shown that in terms of erosion of the skull base, the most common site is the base of the sphenoid bone, followed by pterygoid process, then clivus and petrous apex (Figure I.51,B) (Li *et al*.,2013; Liu *et al*.,2009). The explanation could be that these anatomical sites have no tissue barrier and are very close to the original

tumor (Li *et al.*,2013). The evolution of NC is gradual, starting from the proximal areas of the nasopharynx and then spreading to the distance (Li *et al.*,2013).

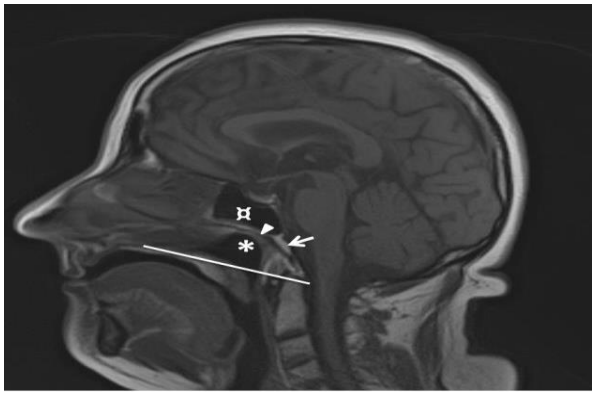

Figure I.49. Sagittal T1- weighted MRI image showing the normal nasopharynx (asterix) and its superior limit represented by basiphenoid (arrowhead) and clivus (arrow). Superiorly is sphenoid body with a large sphenoid sinus. Inferiorly, the junction between nasopharynx and oropharynx (white line) is represented by a line between the hard palate and anterior arch of C1 vertebra (Turliuc *et al*.,2016c).

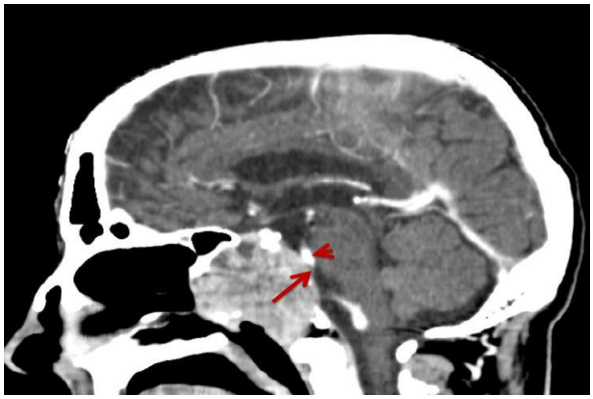

Figure I.50. Sagittal contrast-enhanced CT image in which tumoral invasion of prepontine cistern (arrow) with basilar artery compression (arrrowhead) is seen (Turliuc *et al*.,2016c).

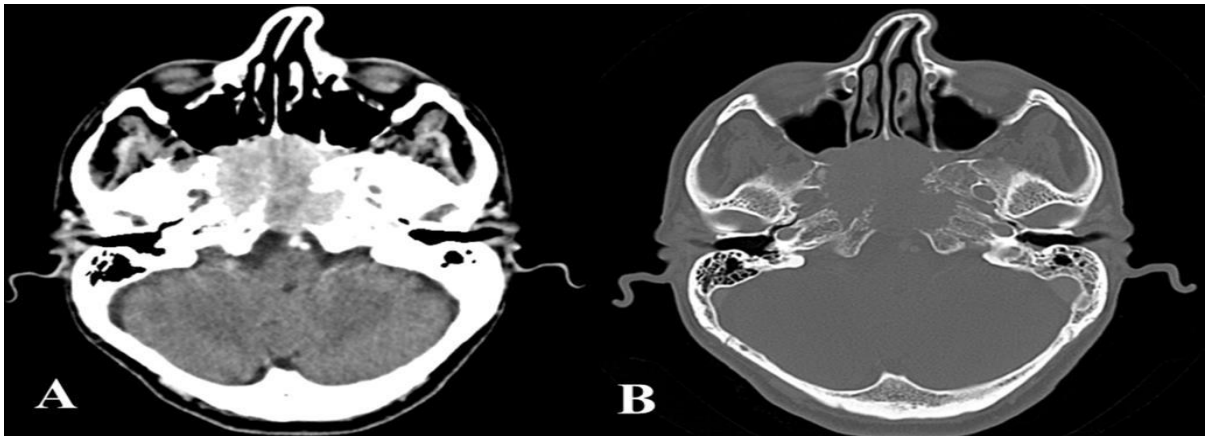

Figure I.51. Axial contrast-enhanced CT images: A. Hyperdense tumor is seen (captures the contrast substance) invading the skull base; B. Bone window CT images show bone destruction of skull base (Turliuc *et al*.,2016c).

Conclusions

Poor clinical symptoms of NC do not alert the patient, so it progresses insidiously to gigantic sizes invading the base of the skull. Knowledge of NC ways of expansion is important for early diagnosis and the understanding of tumor behavior by radiologists, ophthalmologists, neurosurgeons and oncologists is essential in order to provide treatment more rapidly for the patients (Turliuc *et al.*,2016c).

I.3. Inflammatory ophthalmology

I.3.1. Researches regarding chronic dacryocystitis in adult patients in Moldova region, Romania

Background

Lacrimal drainage system's disorders account for only 3% of all visits to Ophthalmology clinics (Chakrabarti *et al.*, 2016). Dacryocystitis is the inflammation of the lacrimal sac (LS) and the nasolacrimal duct (NLD). The aetiology of this disease involves the micro-organisms which spread from the eye conjunctiva or the nasal mucosa. It is a frequent lesion of the lacrimal system associated with the anatomical or functional obstruction of the NLD. The consequences are the distension of the LS due to the accumulation of mucous and tear secretions (Fay and Dolman, 2016). Specimens of dacryocystectomy and dacryocystorhinostomy (DCR) sent by the ophthalmologist for the histo-pathological examination commonly allow specialists to set a diagnosis of non-granulomatous inflammation of the LS, malignant or benign epithelial tumours that could be rarely identified and lymphoid tumours (Anderson *et al.*, 2003; Heindl *et al.*, 2010). However, dacryocystitis is the most common inflammatory lesion of the LS, representing 79–87% of all pathologies at this level (non-tumoral and tumoral) (Anderson *et al.*, 2003; Marthin *et al.*, 2005). Nasolacrimal duct obstruction (NLDO) very often impedes eye quality and the comfort of life of any patient. Thus, chronic dacryocystitis (CD) may induce acute dacryocystitis (AD) and generate complications such as orbital cellulitis, keratitis, and other very severe ocular complications (Mcewen, 1997; Cazzavillan *et al.*, 2010; Ali, 2015). This medical condition is a contraindication for surgeries like: glaucoma, cataract, and other intraocular surgeries (Rabina *et al.*, 2013). To improve the quality of life and eye comfort of patients, treatment and prevention of lacrimal system pathology are necessary. Nowadays, surgery is the most important therapeutic procedure for treating NLDO. It has been previously reported that, for congenital NLDO in children older than 1 year of age, the success rate of probing lacrimal passage within 6 months is only 47.4% (Al-Faky and Mousa, 2015). DCR has reported to offer good success rates around 90% with a safety profile in treating NLDO (Coumou *et al.*, 2016). However, it is disturbing that 5–10% of adult patients with NLDO still complain of different degrees of epiphora symptoms following successful DCR and dacriocistectomy treatment (Shams *et al.*, 2014). It is very important to be able to determine the pathophysiological mechanisms specific to NLDO, and to search for new treatment protocols for this disease (Paulsen *et al.*, 2002; Frame and Burkat 2009; Rehorek *et al.*, 2011). The

mucosa of the lacrimal duct system (LDS) plays an important role in local defence mechanisms meant to prevent invasion of pathogenic agents. The first line of defence is the lacrimal duct epithelium; the epithelium is a pseudostratified type with goblet superficial columnar and basal cells, cells that are tightly bound by junctional complexes (Ishikawa *et al*., 2011). In NLDO, dacryocystitis is the accumulation of mucoid secretions and desquamated cells, which is a very good environment for bacterial proliferation. CD usually affects adult patients above 30 years of age, especially women. Persistent epiphora or regurgitation of mucopurulent or mucoid discharge on application of pressure over the LS area represents the clinical signs for CD (Bharathi *et al.*, 2008; Amin *et al.,* 2013). The LS wall samples collected after dacriocistectomy helped us in understanding the pathologic processes specific to NLDO, the cause of dacryocystitis. The most common findings of biopsy specimens of the LS walls are the non-specific inflammatory lesions, which influence disease prognosis in each patient. Based on the degree of chronic inflammation, capillary and fibrosis proliferation, each biopsy specimen was assigned a chronic inflammation score (CIS), which in turn is an important and relevant factor for the prognosis outcome of the patient (Anderson *et al.*, 2009). Currently, dacryocystectomy is rarely used, with indications for LS tumors (Staff, 2017) but also for CD in elderly patients who do not qualify, for DCR or refuse dacryocystectomy. I published a clinical study in which was analized from an epidemiological and histopathological point of view various changes in the LS in CD and also was evaluated the chronic inflammatory score (CIS) of CD in patients in Moldova region, Romania, between 1999-2015. This disease had not been studied in our country and so we wanted to highlight the changes in the histopathological aspects that occurred in CD, in specimens excised by the ophthalmologist following the operation of dacryocystectomy.

I published also an article regarding the surgical technique of staining the LS in dacryocystectomy. All theese researches data are presented below and they were published in ISI ranked journals.

Costea CF, Dumitrescu GF, Turliuc MD, Dimitriu G, Chihaia MA, Indrei L, Dumitrescu N, Cucu A, Cărăuleanu A, Gavrilescu CM, Costache II. A 16-year retrospective study of dacryocystitis in adult patients in the Moldavia Region, Romania, *Rom J Morphol Embryol*, 2017, 58(2):537–544, IF=0.912

Bogdanici CM, **Costea CF**, Dimitriu G, Chihaia MA, Carauleanu A, Andrei Cucu, Sava A, Dumitrescu GF, Turliuc S, Turliuc MD. Intraoperative identification of lacrimal sac by means of methylene blue, *Revista de Chimie*, 2018, 69(1): 172-174, IF=1.605

Material and methods

This retrospective and descriptive study is carried out over a period of 16 years (between 1999-2015) of patients diagnosed with CD and operated in the Second Ophthalmology Clinic of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iași, Romania and which were registered in the files of the Pathology Department of the same hospital. For each patient, we took into account the demographic data, the location of the lesion and the histopathological changes of the LS, following the dacryocystectomy operation. The specimens were sectioned into 4 μm thick and standard stained fragments with Hematoxylin – Eosin (HE) and in some cases immunohistochemical staining or using the technique EnVisionTM + and anti-cytokeratin (CK) AE1 / AE3 antibody.

The pathologist examined the specimens obtained from the LS, identifying chronic inflammatory cell fibrosis, infiltration and capillary proliferation. A "chronic inflammation score" (CIS) was also established based on the inflammatory cells identified in the examined specimens (Costea *et al*.,2017).

The histopathological features found were graded as follows: (1) The intensity of inflammatory cell infiltration [number of inflammatory cells in a high-power field (HPF)] was considered to be mild: <50 cells, moderate: 50–200 cells, severe:> 200 cells; (2) In our study, the degree of capillary proliferation (number of capillary vessels in a HPF) was reviewed as mild: <5, moderate: 5–10, severe:> 10; (3) Also, the density of fibrosis (the amount of fibrotic tissue in a HPF) was assumed to be mild: <25%, moderate: 25–50%, severe:> 50%. In order to determine the intensity of chronic inflammation in the LS wall, all these three histopathological features were scored individually by their severity (mild = 1, moderate = 2, and severe = 3).

We then calculated a sum, total score for each case we studied, ranging between 3 and 9 and named chronic inflammatory score (CIS). Finally, each specimen was grouped according to its CIS as: mild (CIS <3), moderate (3 <CIS <6) and severe chronic inflammation (CIS> 6) (Costea *et al*.,2017).

Results

768 patients admitted to the Second Ophthalmology Clinic of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital Iași, Romania for a period of 16 years (1999-2015) and from these were selected only those who were diagnosed with CD and operated by dacryocystectomy (18 patients - 2.34% of cases) (Figure I.52).

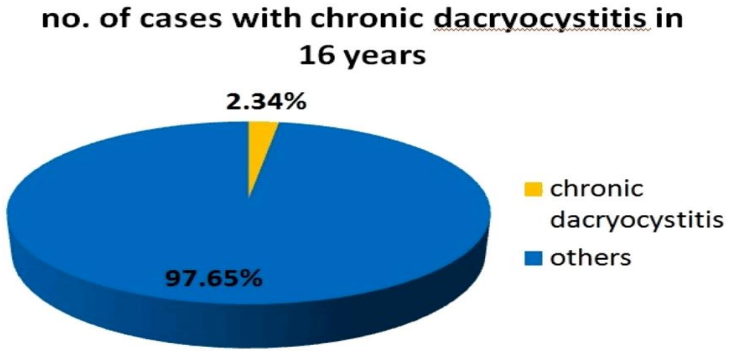

Figure I.52 – Incidence of CD in a total of 768 patients examined over a 16-year period (1999–2015) in the Department of Pathology, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania (Costea *et al.*,2017).

The number of patients diagnosed with CD annually was small, but it was found that since 2002 there has been an unsystematized increase (Figure I.53).

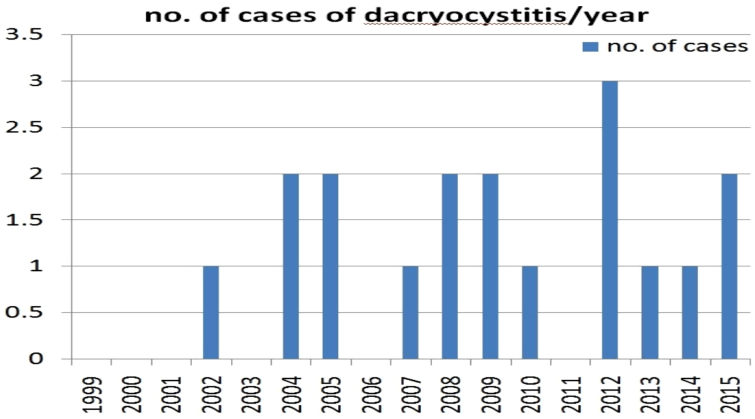

Figure I.53 – Number of CD cases per year over a 16-year period (1999–2015) (Costea *et al.*,2017).

The ratio of women to men in our study was 8:1 (Figure I.54) with a median age of 66.27 years (between 33 and 83 years); out of the 18 cases diagnosed with CD, 55.55% were in the 8th and 9th decades of life (Figure I.55).

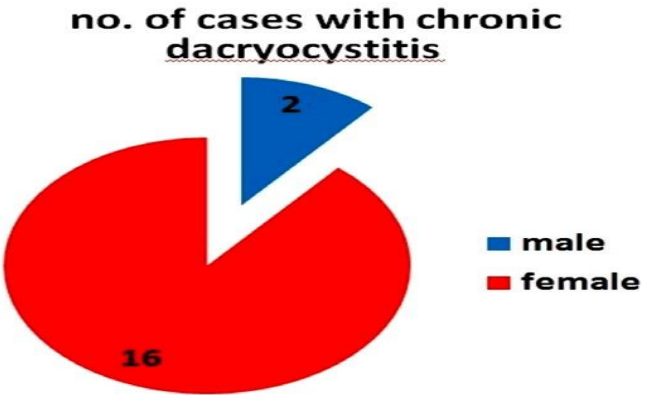

Figure I.54 – Number of CD cases correlated with patients'gender (Costea *et al.*,2017).

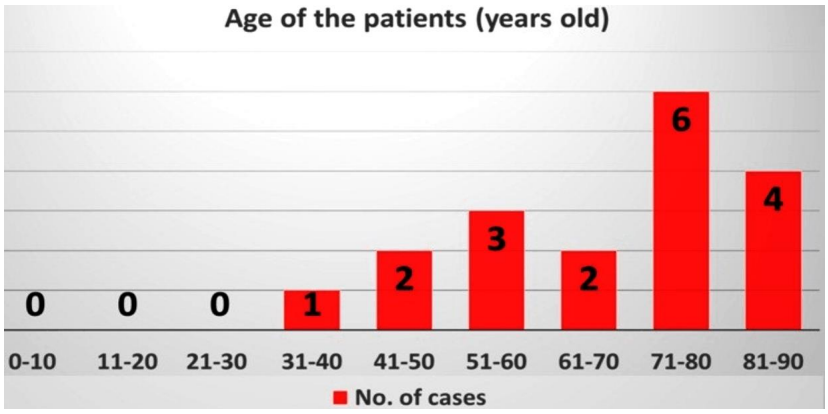

Figure I.55 – Number of CD patients correlated with their age (years old) (Costea *et al.*,2017).

In our case, the LS was affected in 14 patients (77.77%), and the right only in 4 cases (22.23%) (Figure I.56).

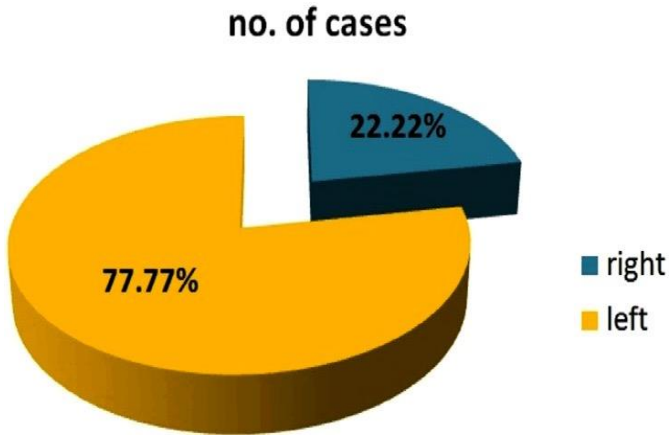

Figure I.56 – Percentage of CD cases correlated with lesion location (Costea *et al.*,2017)

From a clinical point of view, the ophthalmological examination revealed in these patients a round, red and painful inflammatory lesion on palpation located at the level of the medial canthus of the eyelid and at the pressure of the LS, the expression of mucopurulent secretions was observed, being clinically diagnosed and operated for CD, (Figures I.57 and I.58) (Costea *et al.*,2017).

Histopathological aspects revealed important changes in the lumen of the LS, the lamina propria and its epithelium.

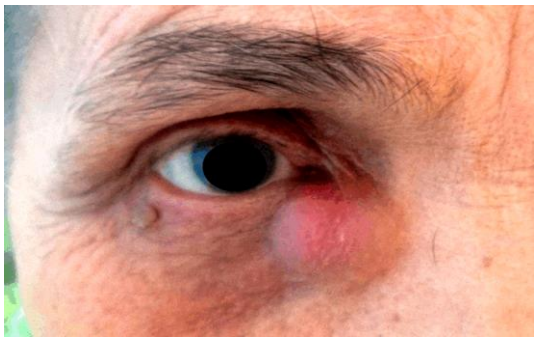

Figure I.57 – CD – preoperative aspect of the patient: round and red swelling localized below the right medial canthal area (Costea *et al.*,2017).

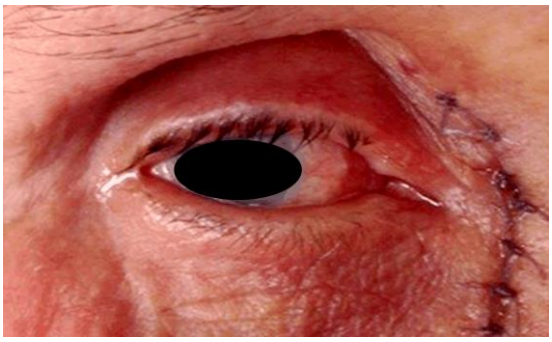

Figure I.58 – CD – postoperative aspect of the patient (Costea *et al.*,2017).

Lumen, mucus, detached epithelial cells, numerous inflammatory cells, and hemorrhage were identified in 77.77% of cases. In 22.22% of cases the lumen of the LS was narrowed, irregular due to inflammatory changes.

We identified significant pathological changes in the epithelium of the LS in 77.77% of cases, epithelial hyperplasia was also found, forming 8-10 layers with numerous goblet cells, developing pseudo-papillary aspects, metaplasia mucosa and in 11.11% of cases the formation of invaginations in the lamina propria was found, similar to the Henle glands (Figures I.59 and I.60) (Costea *et al*.,2017).

Only in 16.16% of cases were metaplasia or necrosis with partial denudation of the tissue (33.33%) .

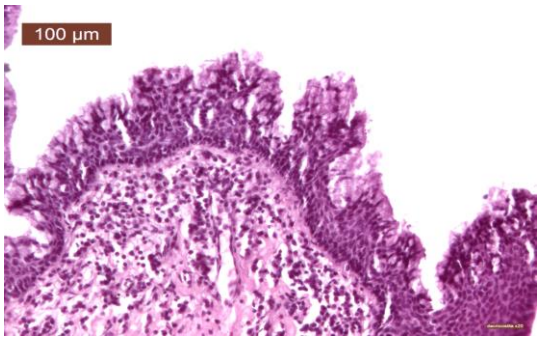

Figure I.59 – CD. Epithelial hyperplasia, including goblet cells, produced pluristratification with 8-10 layers (HE staining, ×200) (Costea *et al.*,2017).

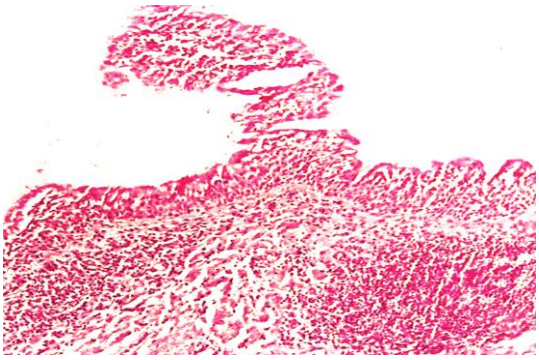

Figure I.60 – CD. Hyperplastic epithelium formed pseudo papillae that protruded into the LS lumen (HE staining, ×100) (Costea *et al.*,2017).

These aspects were found in different parts of the same lesion. In the walls of the LS, inflammatory cells were present (Figure I.61), and newly formed capillaries proliferated. In 5.55% of cases, a low number of reduced collagen fibers and several lymphocytes were reported (Figure I.62). In 3 cases a mild inflammatory infiltrate was identified (16.66%); in 5 cases the inflammatory infiltrate was moderate (27.77%) and severe in 10 cases (55.55%).

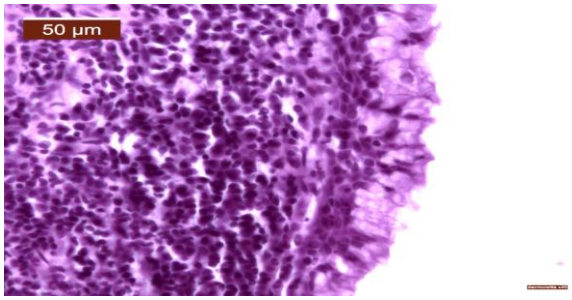

Figure I.61 – CD. Mild inflammation of the LS wall (HE staining, ×400) (Costea *et al.*,2017).

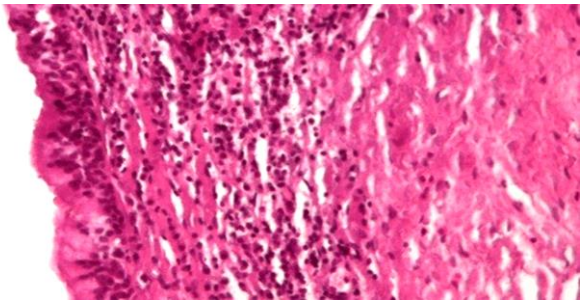

Figure I.62 – CD. Severe infiltration of lamina propria with lymphocytes (HE staining, ×400) (Costea *et al.*,2017).

The proliferation of newly formed capillaries was severe, in only 2 cases (11.11%) mild in 5 situations (27.77%) and moderate in 11 cases (61.11%). Fibrosis was identified in 2 patients as severe (11.11%), mild in 11 patients (61.11%) and moderate in 5 patients (27.77%) (Costea *et al.*,2017).

Regarding CIS, in 13 patients it was moderate (72.22% of cases), one patient had a CIS score < 3, meaning mild inflammation and a CIS score > 6, in 4 patients (22.22%), representing severe inflammation changes. The severity of histopathological changes is illustrated in Table I.5. and CIS is highlighted in Table I.6 (Costea *et al.*,2017).

Table I.5 – Gradation of histopathological features discovered in lamina propria of lacrimal sac wall (Costea *et.al.*,2017)

Intensity of histopathological features	Inflammatory cell infiltrate (*n*=18 cases)	Fibrosis (*n*=18 cases)	Capillary proliferation (*n*=18 cases)
Mild (=1)	3 (16.66%)	11 (61.11%)	5 (27.77%)
Moderate (=2)	5 (27.77%)	5 (27.77%)	11 (61.11%)
Severe (=3)	10 (55.55%)	2 (11.11%)	2 (11.11%)

Table I.6 – Dacryocystitis cases distribution based on chronic inflammation score (Costea *et.al.*,2017)

Chronic inflammation score (CIS)	No. of cases (%)
Mild (<3)	1 (5.55%)
Moderate (3<CIS<6)	13 (72.22%)
Severe (>6)	4 (22.22%)

Discussions

Although the number of cases diagnosed with CD included in our study was small, it was found that for 16 years (1999-2015) their number gradually increased in the region of Moldova, Romania, especially in elderly patients (Costea *et al*.,2017). In our study we identified an increased frequency of this disease in the female population in the 8th and 9th decade of life (Costea *et al*.,2017), data found by other authors. Marthin *et al*. (2005) found twice the incidence of female patients with a mean age of 63.6 years compared with male patients with a mean age of 57.8 years (Marthin *et al*., 2005). Our study identified a higher incidence of left than right LS, data reported by other authors. Ramesh Murthy found similar data in his study published in 2011, explaining that the lacrimal fossa forms a larger angle on the right than on the left, which causes a higher incidence on the left side of the CD than on the right side (Ramesh Murthy, 2011).

Histopathological features of CD have been studied since the beginning of the 20th century. In 1925, the ophthalmologist Samuel Hanford McKee published the first scientific paper about the histopathological aspects of CD. He showed in his study that the lumen of the LS was often narrowed and irregular due to inflammatory protuberances, which gave the surface of the mucosa a villous aspect (McKee, 1925). These "finger-like" projections of the lumen of the LS were considered in his work to be the result of the marked lymphocytic infiltration in the lamina propria and granulation tissue. Similarly to our research, McKee also found that the same specimen of CD could present different histological aspects along the wall of the same inflamed LS (McKee,1925). We found it interesting that in the 8 cases that he reported there were no cases of squamous metaplasia of the epithelium (McKee,1925), which we have found in almost a quarter of our patients (Costea *et al.,* 2017).

This aspect appeared only in one paper published by Prasad *et al*. (Prasad *et al*.,1958). In our study we found epithelial changes in the LS, but also chronic inflammation which was in some cases even severe (Costea *et al*.,2017). Epithelial hyperplasia caused the formation of 8-10-layer finger-like projections on the LS lumen and goblet cells, which formed invaginations similar to Henle's glands (Mc Kee, 1925; Tucker *et al.,* 1997; Anderson *et al*., 2003; Chakrabarti *et al*., 2016; Costea *et al*., 2017). Regarding the pathological mechanism of CD, some researchers presumed that intraluminal exudate could be responsible for squamous metaplasia of the remaining epithelium of the LS due to chronic irritation and for ephithelial necrosis (Schaefer, 2015).

The intraluminal content irritation upon the epithelial lining of the LS can determine adaptive mechanisms that may produce the substitution of epithelial cells that are sensitive to

stress by squamous cells, which are capable to withstand an adverse environment (Schaefer, 2015). Therefore, the squamous metaplasia of the columnar epithelium appeared in the LS epithelium in response to chronic irritation, due to inflammation; however, although metaplasia is not directly carcinogenetic, if it persists it is a predisposing factors to metaplasia, it may induce malignant transformation in metaplastic epithelium of the LS wall (Costea *et al*.,2017).Therefore, more cases of could develop with malignancies of the LS, if patients do not go to the ophthalmologist to get early treatment for CD and also in other cases of periocular and orbital diseases (Indrei *et al*., 2010).

However, some authors mention that LS specimens after dacryocistectomy frequently show inflammation in 79% to 98% of the cases (Tucker *et al*., 1997; Marthin *et al.*, 2005). Non-granulomatous inflammation was identified in 85.1% of the cases, while granulomatous inflammation (sarcoidosis) was only detected in 2.1% of the cases (Marthin *et al.*, 2005). In our study, only non-specific chronic inflammation was identified (Costea *et al*.,2017).

According to previous clinical studies, the lack of epithelial cells, destruction of blood vessels and fibrosis of the connective tissues of the lamina propria may impair the function of the tears out-flow and induce dacryocystitis (Yang *et al.*, 2018).

According to recent studies, inflammation from of the anterior segment of the eye or nose determines oedema of the mucous membranes, destroying the connective tissue fibers arrangement with reactive hyperaemia, and then induces temporary blocking of the lacrimal canals, with chronic infections, occurs ultimately complete fibrous obstruction of the lumen of the efferent lacrimal duct (Hou *et al.*, 2017). According to previous clinical studies, the lack of epithelial cells, destruction of blood vessels and fibrosis of the connective tissues of the lamina propria may impair the function of the tears out-flow and induce dacryocystitis (Yang *et al.*, 2018).

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Specialized papers underline the fact that anatomo-pathologists should identify inflammatory changes in all CD cases. They should also establish the CIS because it is

important for therapy to be applied based on these scores (Ozer *et al*., 2012; Chakrabarti *et al*., 2016). On the other hand, the presence of vascular proliferation, severe lymphocytic infiltration and fibrosis signify late stages of the disease (Costea *et al*.,2017).

Conclusions

Early treatment of CD by an ophthalmologist is essential. In the region of Moldova, Romania, this pathology is frequently reported in elderly women, in the 8th and 9th decade of life, probably due to poor socio-economic conditions and due to a deficient immune system. The histopathological aspects of LS specimens differ from patient to patient. Histopathological changes in CD may indicate several adoptive aspects, which may in some cases lead to malignant changes in the LS. That is why the early diagnosis of CD by ophthalmologists is important, avoiding the occurrence of possible complications (Costea *et al*.,2017).

I.3.2. Clinical studies regarding the use of methylene blue in ophthalmic surgery in chronic dacryocystitis

Background

Methylene blue (MB) has many uses in medical practice, being used in various forms for the treatment of infections, malaria, methemoglobinemia and dye for cells, tissues and bacteria (Figure I.63) (Bogdănici *et al*.,2018).

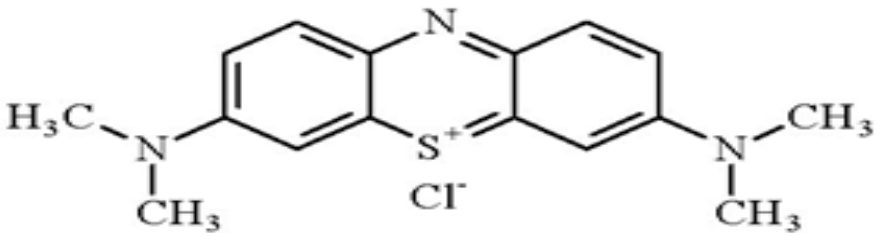

Figure I.63 - $C_{16}H_{18}ClN_3S$. Relative molecular mass (anhydrous form): 319.85 (PubChem, National Center for Biotechnology Information)

MB was synthesized in 1876 by Heinrich Caro in a German textile factory, and it was used as a dye for textiles (Cuttmann *et al.*,1891; Oz *et al.*,2011; Suwavarusk *et al*.,2015). After 15 years it was first introduced in medicine as an antiseptic in the treatment of malaria (Cuttmann *et al.*,1891) and its use has been extended to diagnostic and therapeutic procedures (Oz *et al*.,2011; Barbosa *et al*.,1971; Wainwright *et al*.,2022; Disanto *et al*.,1972; Hainarosie *et al*., 2017a; Hainarosie *et al*., 2017b). Dacryocystectomy is an ophthalmologic operation that represents the complete surgical extirpation of the lacrimal sac, first described in 1724 by the English ophthalmologist John Thomas

Woolhouse (Toti,1904), being a surgical procedure of choice before the introduction of dacryocistorinostomy (DCR) by the Italian physician Addeo Toti, in the early twentieth century for the treatment of dacryocystitis and lacrimal fistulas (Ali, 2014).This study aims to present two cases of CD, where MB is used to stain the lacrimal sac during the dacryocystectomy operation and it was published in an ISI ranked journal.

Material and methods **-** In elderly patients with associated high anaesthetic risk, dacryocystectomy is an alternative to DCR. This is an observational clinical study for two cases of CD where dacryocystectomy combined with MB (1%) staining of lacrimal sac was performed.

Clinical case No.1

An 81-year-old female patient was admitted into the 2nd Ophthalmology Clinic of the "Prof.Dr.Nicolae Oblu" Emergency Hospital of Iași, Romania complaining of recurrent epiphora, muco-purulent discharge in both eyes and inflammatory oedema of the skin surrounding the lacrimal sac for the past six months. The patient is known with atherosclerosis, arterial hypertension, lower limb varices and pulmonary fibrosis (Bogdănici *et al.*,2018).

Clinical Case No.2

A 74-year-old female patient with hypothyroidism was hospitalized in the same clinic of the same hospital, because she presented chronic epiphora, lower eyelid and perisacular (purple-red) oedema of the left lacrimal sac area, which developed in the last ten months (Bogdănici *et al.*,2018).

In both cases, patients were treated prior surgery with local and general large spectrum antibiotics and with steroidal anti-inflammatory medication. The symptoms remitted partially after the treatment, but reoccurred later. In the first patient, a high anaesthetic risk was taken into consideration, while the second patient refused the DCR, after this procedure was explained to her. In these circumstances, dacryocystectomy under local anaesthesia was performed in both patients. The inferior lacrimal punctum was dilated by a punctum dilator. One mL of MB (1%) was flushed in the lacrimal sac using a cannula through the inferior lacrimal punctum (Figure I.64 a).

Skin and subcutaneous tissue were incised until the reflected tendon of the medial palpebral ligament (1.5 cm) was exposed. The lacrimal sac stained by MB was easily identified, dissected from the bone wall of its fossa and sectioned at the isthmus level (Figure I.64 b).

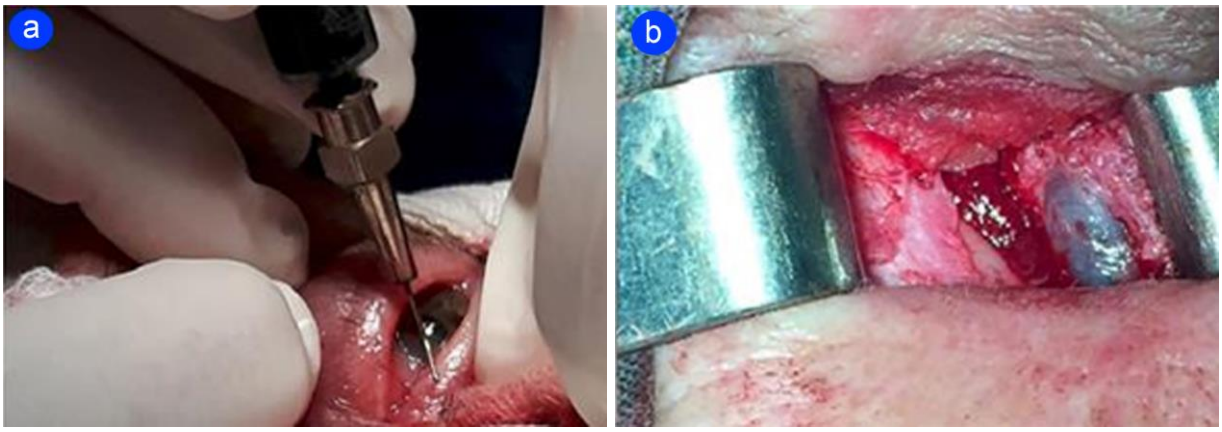

Figure I.64 - Photographies of our oculoplastic surgery. (a) MB (1%) was flushed in the lacrimal sac through the inferior lacrimal punctum. (b) Easy identification of the blue lacrimal sac during dacryocystectomy (Bogdănici *et al.*,2018).

The wound was closed in layers with an interrupted absorbable 6.0 suture, antibiotic was applied, and bandage. The specimens were sent to the Pathology Laboratory of the same hospital for histopathological examination (Bogdănici *et al.*,2018).

Results and discussions

The use of MB during dacryocystectomy surgery is useful to stain the lacrimal sac and in order to be easily identified intraoperatively when it is highly inflamed. In the first clinical case presented, it was observed that the lacrimal sac was located below the medial eyelid ligament. In the second patient, the lacrimal sac was very large (7.5 / 14 mm), being very brittle, due to the chronic inflammatory process. Histopathological examination in the first clinical case revealed hyperplasia of the lacrimal sac epithelium, its lymphocyte infiltration, abundant lymphoid follicles, but also capillary hyperemia under the epithelium (Bogdănici *et al.*,2018). In the second clinical case, the histopathological examination revealed a polypoidal development of the lacrimal sac walls, accentuated inflammation with lympho-plasmocytic infiltrates and hyperemia of the new formed vessels in the sub-mucosal layer and hyperstratification of the lacrimal sac epithelium with several goblet cells in certain areas. In both cases the histopathological diagnosis was chronic non-granulomatous, non-suppurative dacryocystitis (Figure I.65, a and b) (Bogdănici *et al.*,2018).

Postoperatively, the patients did not have any complications, only an average epiphora, which did not cause them any problems (Bogdănici *et al.*,2018). MB has been used in various surgical procedures, especially for the dissection of anatomical structures (Peek *et al.*,2016), but also for the intraoperative marking of the sentinel lymphnode in breast cancer (Vulthalurur *et al.*, 2013; Weingartner *et al.*,1999).

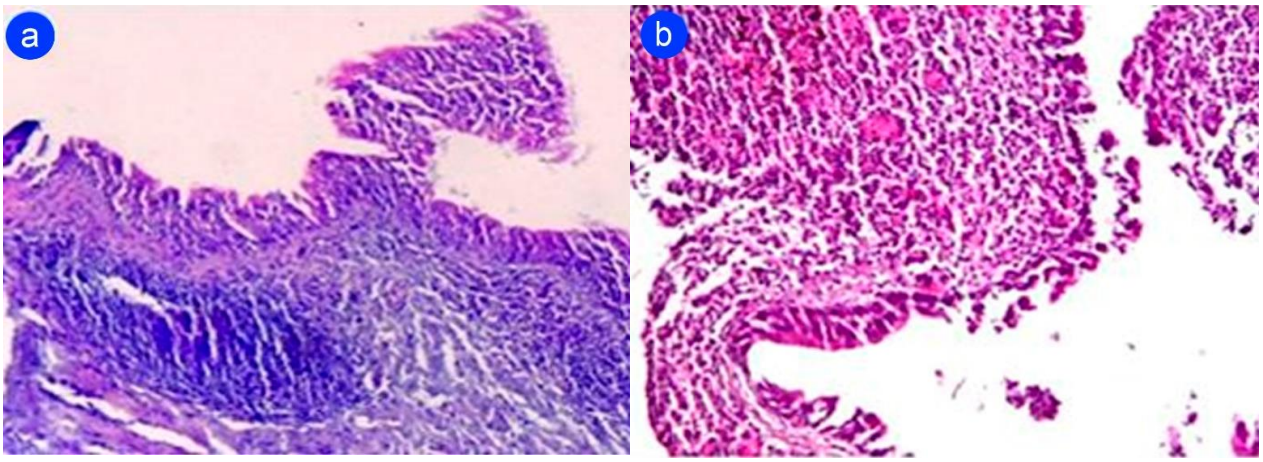

Figure I.65 - Microphotography of the histological specimens. (a) Case no.1. Chronic follicular dacryocystitis (Hematoxylin – Eosin staining, x 200 original magnification). (b) Case no.2. Chronic non-suppurative nongranulomatous dacryocystitis (Hematoxylin – Eosin staining, x 200 original magnification) (Bogdănici *et a*l.,2018).

The risk of anaphylaxis at the local injection of MB is 0.6%, but no such incidents have been reported to date (Vulthalurur *et al.*, 2013) Clinical and experimental studies have not clearly indicated the dose of MB that would cause side effects, but it is known that doses higher than 7 mg / kg body weight may be associated with severe methemoglobinemia and changes in lung functions (Gachot *et al*.,1995; Goluboff,1961).

Local injection of MB during dacryocystectomy does not involve high doses of MB and does not expose the patient to intoxication or other side effects. In surgical practice, MB is used to dye various anatomical structures and to facilitate their precise, and easy dissection (Peek *et al*.,2016).

The most common uses for MB have been in endoscopic polypectomy (Kondal *et al.*,2014), chromoendoscopy (Giuliano *et al*.,1994; Macovei *et al*., 2014), and intraoperative sentinel node marking in early-stage breast cancer.

In the two clinical cases of CD presented, we used MB (1%) to dye the lacrimal sac intraoperatively, in dacryocystectomy.

Patients had no intraoperative or post-operative systemic complication, only regurgitation of the dye upon injection through the lower lacrimal punctum which required washing it with Balance Salt Solution (BSS) to prevent corneal and conjunctival staining (Bogdănici *et al*.,2018).

In a clinical study, Vuthalurur *et al*. (2013) on 16 patients diagnosed with advanced malignant eyelid tumours, the authors used without any systemic complications to stain the sentinel lymphatic ganglion. In a retrospective study comprising 35 patients, Dorafshar *et al*.

(2010) reported MB use by topic instillations in wounds to facilitate precise surgical debridement and differentiate viable from non-viable tissues (Dorafshar *et al*.,2010). In their retrospective study of 134 patients, Ranjan and Kumar (2016) used MB to irrigate the lacrimal sac during DCR. Therefore, MB is used in medicine in the clinical and experimental therapy, and in surgery because of its low toxicity, pharmacokinetic properties (Oz *et al.,* 2011; Suwanarusk *et al*., 2015; Disanto *et al*.,1972) and cost-effectiveness (Suwanarusk *et al*., 2015; Peter *et al.*, 2000).

Conclusions

Marking the lacrimal sac with MB (1%) to highlight it during the dacryocystectomy operation facilitates its easy identification and dissection, especially when the inflammation is extended to the underlying tissues or when the lacrimal sac has an ectopic position.

Due to the low surgical risks associated with local anesthesia, this technique may be suitable for elderly patients with CD and comorbidities qualified for dacryocystectomy (Bogdănici *et al.*,2018).

I.4. Ocular prosthesis and eye surface reconstruction

I.4.1. Back to senses – ocular prosthesis

Background

The eyes are sense organs with an extremely important aesthetic role in the expression and aesthetics of the face.

The loss of one eyeball or both, after a trauma or by surgery such as enucleation, evisceration or exenteration is a major injury in the facial aesthetics of the patient having a devastating psycho-emotional impact for him.

Acquired anophthalmia, surgical as a result of serious ocular pathologies (intraocular or intra-orbital tumors, oculo-orbital trauma, severe intraocular infections, atrophic globe, painful globe) were a constant concern of ophthalmologists to find therapeutic solutions to reconstruct the remaining cavity (Costea *et al*.,2019b).

From ancient times until now, constant efforts have been made to find the most biocompatible materials for the manufacture of eye prostheses so that the reconstruction of the remaining cavity is not accompanied by complications.

Orbital implants have gradually developed, all with the aim of being inserted into the patient's anophthalmic cavity, in order to regain a satisfactory aesthetic appearance, but with the permanent loss of visual acuity (Costea *et a*l.,2019b).

This research direction had been materialized by publishing the following article:

Costea CF, Bogdănici CM, Cărăuleanu A, Dimitriu G, Sava A, Dumitrescu GF, Turliuc MD, Cucu AI, Ciocoiu M, Dragomir R, Buzduga CM. Updates of Ocular Prostheses. A review of biomaterials and design in anophthalmic socket. *Rev Chim*, 2019b, 70(1): 239-244, IF=1.605.

Materials and Methods

In this study, we highlighted chronologically different types of materials from which the eye prostheses were made, reviewing the English electronic medical literature: PubMed and MEDLINE (Costea *et al.*,2019b). From ancient Egypt, eyeballs were extracted during the mummification procedure, using balls wax or precious stones in the remaining orbital cavity (Sami and Young, 2010). The oldest eye prosthesis was discovered in Iran, on a skeleton of a woman in the years 2900-2800 BC (Moghadasi, 2014). The prosthesis was probably made of bitumen paste and was 2.5 cm in diameter, hemispherical in shape, covered with gold on the surface and in the middle had a central circle surrounded by lines drawn like the sun's rays (Moghadasi, 2014; Yeshwante *et al*., 2015; Mules 1985). Along the way, various materials were invented and manufactured to make orbital implants.

These ranged from metal, rocks, minerals, biological materials, organic materials, animal products, polymeric materials, chemicals, hydrocarbons, fibers or human fat (Sami and Young, 2010; Costea *et al*., 2019b). All these materials have been used so far for the production of orbital implants, most being bioinert porous materials such as hydroxyapatite (HA), aluminium, polyethylene (PE) that allow the growth of fibrovascular tissue at the level of porosity (Baino and Potestio, 2016).

Results and discussions

I.4.1.1. Types of prostheses

In ophthalmic practice there are two types of prostheses: ocular and orbital (Costea *et al.*,2019b). The eye prosthesis replaces the eyeball, being practically an “artificial eye”, and the orbital prosthesis replaces the entire orbital contents (Kumar and Sajian, 2010). At the moment, there are three types of ocular prostheses in use: (1) stock eyes (2) modified stock eyes and (3) custom-fitted eyes (Jayaswal *et al*., 2011).

The fitting of the eye prosthesis can be done through different techniques: direct (external) impression, impression with stock ocular tray, impression with custom ocular tray, impression with stock ocular prosthesis and wax scleral blank (Mathews *et al.*, 2000).

The prosthetic eye should have the following features: oval whitish outer shell, which must imitate the sclera and a central round portion, painted in different colours (blue, brown, green) to look like the iris and a black circle in the middle to imitate the pupil of the other eye (Haug and Andres, 2000). Iris button positioning is very important for the aesthetics of the ocular prosthesis (Figures I.66 and I.67) (Costea *et al*., 2019b). Modern ocular prostheses have evolved till today from simply using glass to different complex types of materials. There are two main groups of materials for the manufacture of the prosthesis: non-integrated (non-porous) and integrated (porous) (Shome *et al*., 2010).

Figure I.66 – The ocularist paints the iris button on the eye's prosthesis (Costea *et al*., 2019b).

Figure I.67 – After the enucleation or the evisceration, the ocularist is fitting the prosthesis, after 6-8 weeks following the surgery (Costea *et al.*, 2019b).

Non-integrated implants (non-porous) **-** The materials used for non-porous prostheses do not allow the growth of tissue in their inorganic structure or the attachment of extraocular muscles (Costea *et al.*,2019b).

If extraocular musculature attaches to the surface of these implants, prosthesis migration may occur and therefore must be coated with donor sclera or polyester to improve its motility (Allen, 1983; Trichopoulos and Augsburg, 2005; Yeshwante *et al.,* 2015). These types of prostheses are made of silicone, glass, and polymethylmethacrylate (PMMA) spheres (Cheeah *et al*., 2004; Shome *et al*., 2010).

Glass **-** In 1885, Mules performed an evisceration and used a glass orbital implant (Mules, 1885). The eye prosthesis was made of hollow glass, very common used during the World War II (Baino *et al.,* 2014). Mules noted also that this type of glass implant had complications in 50-90% in his cases. He observed the extrusion of the glass sphere in his cases (Baino *et al*., 2014; Mules, 1885). The surgical techniques improved and the rate of extrusion was decreased from 21% to 10 % (Guyton, 1948; Culler, 1952). Still in the cases of

glass orbital implants always was the risk of breaking, caused by trauma or implosion, at high temperatures (Baino *et al.,* 2014). Moreover, the implant was brittle, heavy and hazardous (Kaira *et al*., 2014). Glass is not used today for the ocular prosthesis and better materials were found to replace it. In 2018, Baino published a study in which he mentioned a new porous material Ca Si O_3. This type of material presents architectural characteristics, proper to be used as orbital implant material, a promising alternative to existing polymeric or ceramic bioinert orbital implants (Baino, 2018).

Silicone **-** For more than fifty years, silicone was largely used not only for different types of surgical devices, but also for orbital implants, due to its beneficial properties: easy handling, flexibility and chemical inertia (Baino *et al*., 2014). Soll D.B. at the end of the 1960s, proposed an inflatable silicone implant, filled with silicone gel (Soll, 1969; Soll, 1973). This type of material was abandoned because of the complications which occurred, both post-operatory and intra-operatory" (Baino *et al*., 2014). At the end of the 1980s, silicone orbital implants, with non-porous spheres, either bare or wrapped, and extraocular muscle cone centred, attached to the four rectus muscles, were introduced (Baino *et al.,* 2014). Till today silicon orbital implants are used successfully (Costea *et al.,* 2019b).

Polymethylmethacrylate (PMMA) **-** PMMA is an important material in ophthalmology, due to its excellent transparency and biocompatibility with ocular tissues (Baino *et al.,* 2014). PMMA is used today not only for manufacturing the artificial intraocular lenses (Bozukova *et al.,* 2010) or contact lenses (Lloyd *et al*., 2001), but also for orbital implants, (Groth *et al*., 2006) and it is used also in neurosurgery and maxillofacial surgery for extensive orbito-cranial and facial defects after traumas.

PMMA ocular implants appeared in the 1980s, when Frueh and Felker described for the first time, a PMMA sphere in an envelope of sclera (Frueh and Felker, 1976). The complications after PMMA implant were post-surgery edema (Tyers and Collin, 1985), unacceptable pain, implant migration, and extrusion (Leatherbarrow *et al*., 1994) or necrosis of the conjunctiva (Kamal-Siddiqi *et al*., 2008).

Integrated implants (porous) **-** The integrated implants with porous surface proved to be a very good option. They allow the insertion of posts and pegs and fibrovascular ingrowth in depth all over the implant (Guyton, 1948). In the absence of a vascular base, this fibrovascular base ingrowth at the level of the porous implant should: (1) decrease the risk of infection rates after the surgery (2) lead to the increase of the surgical success rate, and (3) reduce the rate of migration or extrusion of the implant over time (Custer *et al.,* 2003; Trichopoulos and Augsburger, 2005; Sami and Young, 2010).These qualities are due to the

porous type of the material of the implant, which helps in anchoring it in much safer conditions (Sami and Young, 2010; Baino *et al.*, 2014), providing blood supply within the implant, and after the surgery reducing the risk of infection (Chalasani *et al.*, 2007).

Bone-derived orbital implants **-** In 1899, Schmidt introduced the orbital implant made of mineral matrix of bovine cancellous bone (Schmidt, 1906). The process of manufacturing this implant was complex and it implied heating spheres of cancellous bone, in order to destroy all organic matter, leaving only calcium phosphate mineral framework behind (Schmidt, 1910; Klement and Tromel, 1932; Bredig, 1933; Baino *et al.*, 2014). These types of implants were used until 1950s, after that implants made of PMMA, and silicone took the place of the bovine bone orbital implants (Baino *et al*., 2014).

Proplast-Teflon **-** Proplast was introduced by Lyall at the end of the 1970s (Lyall, 1976) and it was an inert felt-like composite material. It was composed of polytetrafluorethylene (Teflon) and carbon fibers (Lyall1976). The advantage of proplast was that it could be invaded by fibrous tissue, having the advantage of non-extrusion (Baino *et al*., 2014). Nevertheless, the use of this type of implant has decreased, due to post-surgery complications, such as long-term infections (Whear *et al.*, 1993).

Hydroxyapatite (HA) **-** Coralline porous HA – $Ca_{10}(PO_4)_6(OH)_2$ – was introduced by Perry (Perry 1994) and become the most frequently used material for the manufacture of ocular prosthesis, after primary enucleation (Hornblass *et al.*, 1995). Porous HA was made from a specific genus of reef building-coral. It has similar architecture to human cancellous bone, with interconnecting channels (Sami and Young, 2010).

HA is a primary inorganic portion of human bones and the process by which implants of HA are made from sea coral, involve intense heat, which denatures proteins, in order to reduce the immune response (Sami and Young, 2010). Porous HA allows the ingrowth of fibrovascular tissues in pores, when it is implanted in soft tissues (Perry, 1991). Few studies reported that unwrapped HA does not become encapsulated, like silicone or PMMA spheres (Holmes, 1979; Perry, 1991; Dutton, 1991). Like other ocular prosthesis, HA allows fibrovascular ingrowth reducing the risk of migration, infection and extrusion (Nunnery *et al.*, 1993). Another advantage of the HA is attachment of extraocular muscles, improving safely the implant motility (Perry, 1991; Dutton, 1991). Studies have also mentioned that the rate of vascularization depends on pore dimensions (Bigham *et al.*, 1999). HA stimulates the occurrence of a foreign-body giant cell reaction (Rosner *et al.*, 1992), and in animal models, this reaction can last up to one year from the orbital implant (Sires *et al*., 1995; Saitoh *et al*., 1996).

Polyethylene (PE) **-** PE is a straight-chain, high-density hydrocarbon. It is made by polymerization of ethylene molecules under temperature and pressure (Jung *et al*., 2012). At the end of 1980, porous PE was introduced in the ophthalmic practice. It was considered an efficient and safe alternative to coralline porous HA sphere (Karesh and Dresner, 1994; Jin *et al.,* 2000; Blaydon *et al*., 2003; Chee *et al*., 2013). They were safely used as implants placed in the orbit, without wrapping.

This poly-porous form of PE (Medpor) is made by compacting and heating. PE granules are found in spherical shapes of different size. PE is highly biocompatible material, non-allergenic and non-toxic. It is resistant and permits the surgeon to suture direct the extraocular muscles without the need of sclera (Spector *et al*., 1975; Klawitter *et al*., 1976; Lee *et al.,* 2005).

Many clinical studies showed that PE implants are very good option and with very favorable surgical outcome (Baek, 2000; Lee *et al*., 2000; Kim *et al*., 2002; Blaydon *et al*., 2003; Choi *et al*., 2003). PE implants are associated with less fibrosis and inflammation (Goldberg *et al.,* 1994; Li *et al*., 2001; Jordan *et al.,* 2004). Electron microscopy proved that porous PE implants show a smoother surface than coralline, aluminium oxide or synthetic HA. The main disadvantage of this material is a lower level of vascularization, than that of coralline HA (Jordan *et al*., 2004; Costea *et al.,* 2019b).

Polytetrafluoroethylene (PTFE) **-** Expanded porous polytetrafluoroethylene (ePTFE) prosthesis was studied on rabbit models at the end of the 1990s (Dei *et al*., 1998). In spite the fact that fibrovascularization took place in the implants, some authors reported various degrees of chronic and acute inflammation (Mortemousque *et al*., 2001; Mortemousque *et al*., 2002).

Aluminium oxide **-** Since 1990s aluminium oxide (Al_2O_3), also called alumina was used as orbital implant. Alumina is biocompatible material, well-tolerated, like HA, it allows fibrovascular ingrowth (Morel *et al*., 1998). Cases of aluminium encephalopathy, were initially mentioned in the studies, due to the use of ionocem. The blood samples of patients with Al_2O_3 implants revealed normal levels of aluminium; Al_2O_3 remained bioinert and insoluble in tissues. Alumina implants wrapped in polyglactin mesh, had low extrusion rates. Subsequent studies were more reserved, proving higher extrusion rates in a long-term (Wang *et al*., 2009).

Conclusions

There is currently no perfect orbital implant on the market, each ophthalmologist must find an optimal solution for orbital reconstruction, so that it acquires an aesthetic appearance

as acceptable as possible and has as few complications as possible in the short and long term (Costea *et al*.,2019b).

I.4.2. Ab initio – ocular surface reconstruction

Background

The amniotic membrane (AM) is the innermost vascular layer of the three fetal membranes, completely surrounding the embryo, delimiting the amniotic cavity (Rahman *et al.*, 2009; Mamede *et al*., 2012). Dr. J. W. Davis introduced it in 1910 in reconstructive plastic surgery, using it as a graft in a skin burn (De Rötth, 1940). It was then used in ophthalmic surgery, as a graft in order to cover defects in the cornea, sclera, conjunctiva and as a biological patch in ocular surface diseases, or as a carrier of limbal stem cells (Gomes *et al.,* 2005).The first use in ophthalmic practice of the human amniotic membrane (hAM) was documented in 1940 by De Rötth, who applied it to the ocular surface after a conjunctival burn (De Rötth, 1940).

Conjunctival autografts, oral mucosa, and hAM grafts are used for reconstruction of the anterior ocular segment and / or orbit, providing excellent postoperative cosmetic results (Chaudhuri *et al*.,2012; Paridaens *et al*., 2001; Espana *et al*., 2002; Tiutiuca *et al*.,2016). The exceptional properties of hAMs are: antiscarring, anti-inflammatory, immune-regulatory and antifibrotic and antimicrobial activity (Faulk *et al*.; Hao *et al*., 2000; Bailo *et al*., 2004 Ricci *et al*.,2013; De Rötth *et al*.,1940) were studied and encouraged its use in ophthalmology. One of my study aims to highlight the properties and chemical composition of the hAM and to draw a comparison with the chemical composition of human tears and plasma (Costea *et al*., 2018b). The ultrastructural and structural characteristics of the hAM but also its biological properties recommend it as a natural biomaterial, acting as a matrix for tissue regeneration and restoration of the corneal and conjunctival surface, acting as a substrate for epithelial cell migration, which can grow, differentiate and migrate in favor of tissue regeneration (Ibrahim and Vitresia, 2017). Due to the histological similarities between the hAM and the ocular conjunctiva and the cornea, in terms of the composition of collagen, fibronectin and laminin it has been used in the treatment of many ophthalmic diseases such as: corneal ulcer, corneal perforations, heat and chemical corneal-conjunctival burns, pterygium, infectious or vernal keratitis, bullous keratopathy (Hao *et al*.,2000; Chopra and Thomas, 2013; Tehrani *et al.*,2021; Rahman *et al.,* 2009; Șapte *et al*.,2017). The hAM is used cryopreserved at -80^{0}C, fresh or dried, γ-sterilized, denuded membranes (Utheim *et al*.,2018). Fresh hAM contains high levels of growth factors compared to the denuded one (Utheim *et al*.,2018; Cooke *et al*.,2014). Crosslinking of the hAM is necessary to increase its thermal and mechanical

stability for epithelial cell culture, including glutaraldehyde, carbodiimide and UV radiation crosslinking (Fujisato *et al*.,1999).

In particular, with respect to the UV crosslinking procedure, it was demonstrated that the biostability of collagenous tissue strongly depends on the number of crosslinked structures, which are closely related to the UV exposure time (Cavalu *et al*.,2021). Optimal crosslinking of collagen is essential for collagen binding to its receptors. The matrix permeability is drastically affected by the number of crosslinks per unit mass of the photo-crosslinked of hAM (Lai, 2014).

Therefore, hAMs' proper preparation, preservation and clinical application are crucial for the best outcomes in the treatment of different severe ocular disorders. The use of post-operative antibiotics after the application of the amniotic membrane graft influences its structure and properties, in the structure of collagen and its proteins structure (Roiu *et al*.,2020). Also, exposure to UV radiation after eye surgery may influence it's the success of the surgery, as it is known that UV affects the structure and mechanical properties of collagen fibrils (Jariashvili *et al*.,2012).

Few of the histological, chemical and ultrastructural aspects of the hAM researches I published them together with my collegues in articles published in ISI ranked journals and presented below:

Șapte E, **Costea CF**, Cărăuleanu A, Dancă C, Dumitrescu GF, Dimitriu G, Chihaia MA, Buzdugă CM, Cucu A, Turliuc MD. Histological, immunohistochemical and clinical considerations on amniotic membrane transplant for ocular surface reconstruction, *Rom J Morphol Embryol*, 2017, 58(2): 363–369, IF=0.912.

Costea CF, Scripcariu IS, Dragomir R, Dimitriu C, Turliuc MD, Dumitrescu GF, Dumitrescu N, Vornicu V, Sava A, Cucu A, Turliuc S, Carauleanu A. Chemical Properties of Human Amniotic Membrane for Potential Opthalmological Use, *Revista de Chimie*, 2018b, 69(6): 1566-1569, IF=1.605.

Cavalu S, Roiu G, Pop O, Petricas Heredea DA, Costea TO, **Costea CF**. Nano-scale modifications of amniotic membrane induced by UV and antibiotic treatment: histological, AFM and FTIR spectroscopy evidence, *Materials*, 2021, 14(4): art. no 863, IF=3.057.

I.4.2.1. Histological, immunohistochemical and clinical considerations on amniotic membrane transplant for ocular surface reconstructions

Comparative histology: bulbar conjunctiva versus amniotic membrane transplant (AMT) - The ocular bulbar conjunctiva is a transparent, pink, vascularized membrane that covers the eyeball to the sclero-corneal limb. Histologically it consists of two important

layers: the epithelium and the stroma. The mucous surface of the ocular bulbar conjunctiva is richly vascularized with blood and lymph. In the sclero-corneal limbus, the conjunctival epithelium continues with the corneal epithelium (Sanford-Smith, 2001; Sava *et al.*, 2015).

The stroma of the bulbar conjunctiva contains connective tissue, being made up of two layers: 1. the adenoid layer, which consists of reticular connective tissue, with lymphocytes containing follicle-like structures without germ centers (Figure I.68); 2. a deep fibrous layer, which consists of connective tissue with elastic fibers and collagen. The stromal conjunctiva contains nerves and glands (Sava *et al.*, 2015).

The basement membrane of the human amniotic membrane (hAM) is very similar in histological structure to that of the conjunctiva and cornea, especially in terms of collagen content (Malhotra and Jain, 2014).

This feature is important in eye diseases that require reconstruction of the bulbar conjunctiva as well as the cornea.

Conjunctival grafts due to vascularization can contribute to the healing of severe corneal ulcers, they can also be used in the reconstruction of outstanding defects, after excision of the pterygium, conjunctival tumors and corneal injuries (Sanford-Smith, 2001).

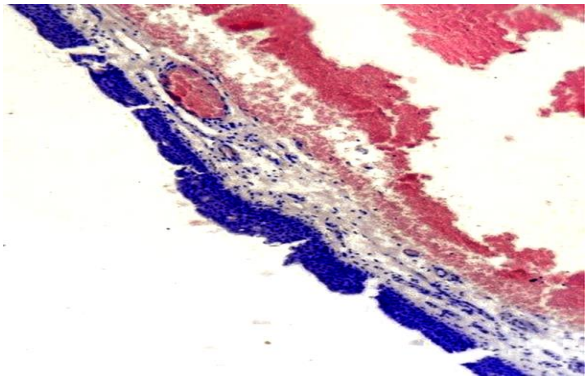

Figure I.68 – Microscopic aspect of eye bulbar conjunctiva: stratified squamous epithelium situated on top of the stromal adenoid layer [Hematoxylin–Eosin (HE) staining, ×100] (Șapte *et al.*, 2017).

Figures I.69 and I.70 illustrate the histological structure of the AM, consisting of a maternal component (the deciduas) and a chorionic plate.

The two components are connected by chorionic villi and are attached together by the cytotrophoblastic shell of the chorionic sac to the decidua basalis (Niknejad *et al*., 2008).

There are no blood vessels, nerves or lymphatic vessels in the AM structure. Its oxygenation is done by chorionic fluid, fetal surface vessels and amnion fluid (Toda *et al.*,2007).

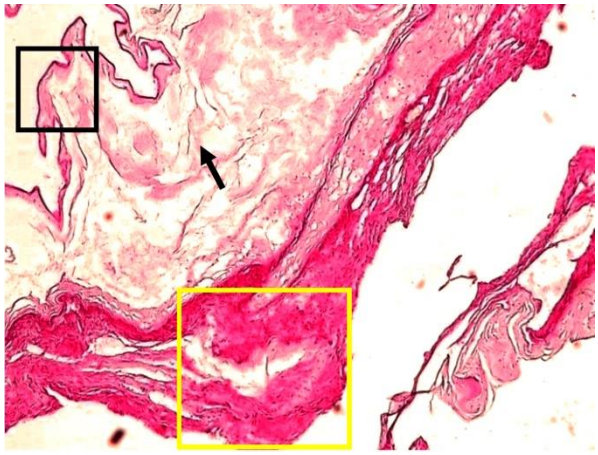

Figure I.69 – The AM of the human is separating the developing fetus from his/her mother "in utero". Black square: AM or fetal side is composed by a single layer of cuboidal epithelial cells, basement membrane and stromal layer. Yellow square: Maternal deciduas made up of trophoblasts situated on a thick basement membrane, and a stromal layer. Black arrow: Between AM and chorionic layer, there is the intermediate layer composed of mesechyme showing fibroblasts (HE staining, ×100) (Șapte *et al.*, 2017).

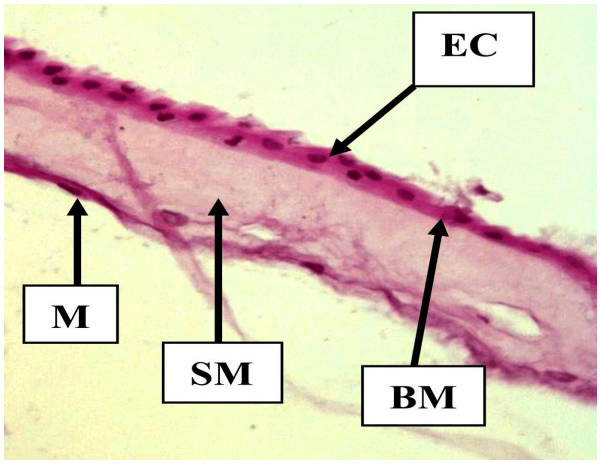

Figure I.70 – Magnified photo of the black rectangle frame from the previous figure, showing the microscopic structure of AM (HE staining, ×400).EC: A single layer of cuboidal epithelial cell with microvilli; BM: A very thick basement membrane; SM: Avascular stromal matrix; M: Mesenchymal intermediate layer composed of fibroblasts (Șapte *et al*., 2017).

Comparative immunohistochemical phenotypes: eye conjunctiva versus amniotic membrane (AM) **-** AM is freshly obtained from donor mothers who have been selected for cesarean section and who have been repeatedly tested serologically during pregnancy for hepatitis B and C virus, immunodeficiency virus, and syphilis (Kim and Tseng, 1995; Shimazaki *et al*., 1997).

AM is an extremely valuable tissue used for eye transplantation due to the similarity of the histological components with those of the basement membrane of the bulbar conjunctiva and cornea (Șapte *et al*.,2017). AM secretes epidermal growth factor (EGF), cytokines, fibroblastic grow factor (FGF), which play an important role in promoting epithelial adhesion, cell migration and differentiation (Lee *et al*., 2006; Wolbank *et al.*, 2009; Yang *et al.*, 2015).

Staining of immunohistochemical AM fragments and bulbar ocular conjunctiva shows that both express immuno-positivy for CK AE1 / AE3 and CK19 bringing evidence that AM fragments can be successfully transplanted to the ocular surface to complete conjunctival defects (Figures I.71 - I.74) (Șapte *et al*., 2017).

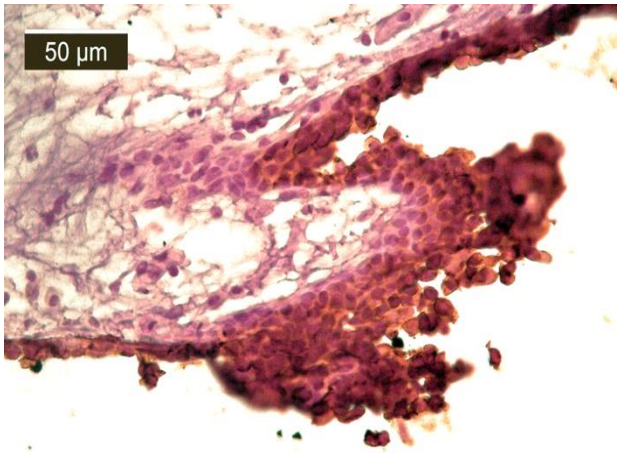

Figure I.71 – Bulbar conjunctiva: epithelial cells shows immunopositivity for CK AE1/AE3 (Immunostaining, anti-CK AE1/AE3 antibody, ×400) (Șapte *et al*., 2017).

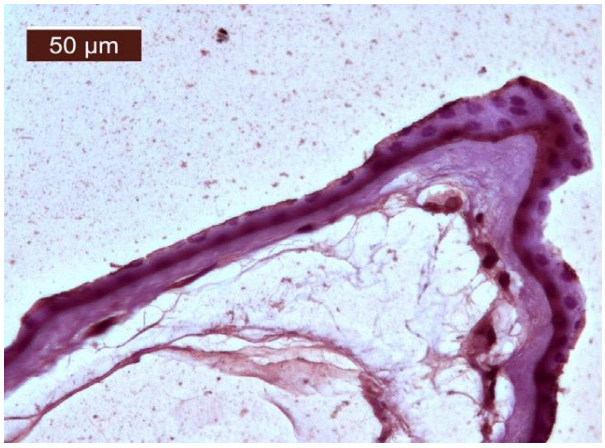

Figure I.72 – AM: epithelial cell express CK AE1/AE3 (Immunostaining, anti-CK AE1/AE3 antibody, ×400) (Șapte *et al*., 2017).

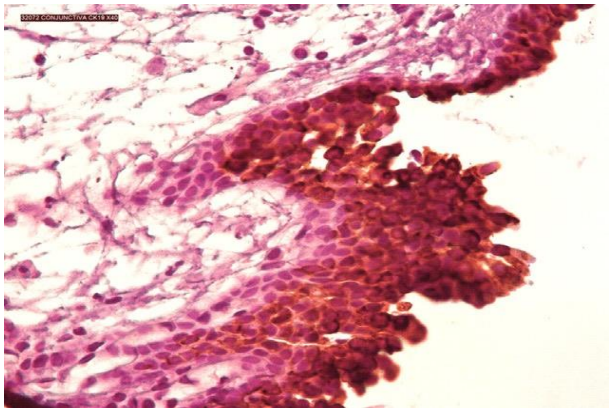

Figure I.73 – Bulbar conjunctiva: epithelial cells show immunopositivity for CK 19 (Immunostaining, anti-CK 19 antibody, ×400) (Șapte *et al.*, 2017).

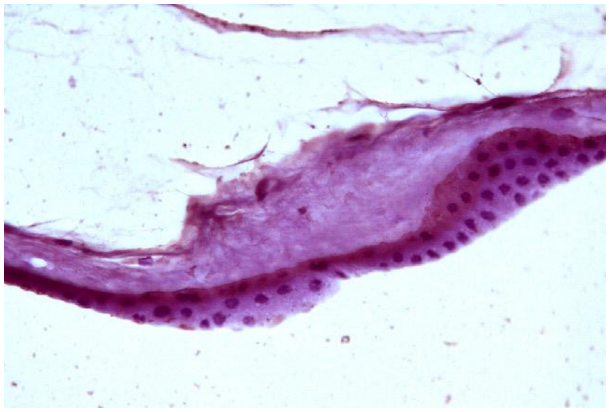

Figure I.74 – AM: epithelial cells express immunopositivity for CK 19 (Immunostaining, anti-CK 19 antibody, ×400) (Șapte *et al*., 2017).

It has been reported in the literature that hAM can transform its own cells into epithelial-like conjunctival cells and create an efficient microclimate for amniotic epithelium differentiation (Giasson *et al*., 2006; Wang *et al.*, 2006; Fatima *et al.*, 2008; Yang *et al*, 2015).

Although the mechanism of action of hAM is not fully understood, it is accepted that this mechanical substrate helps the migration of epithelial cells and their attachment to corneal defects, favoring ocular reconstruction (Malhotra and Jain, 2014).

AM procurement, processing and preservation for ocular transplantation

Kim and Tseng mention how to procure the human placenta in strictly aseptic conditions during a cesarean section and then it has to be rinse in BSS mixed with antibiotics cocktails (50 μg / mL streptomycin, 50 μg / mL penicillin, 100 μg / mL neomycin and 2.5 μg/ml amphotericin B) in sterile conditions (Kim and Tseng, 1995; Tseng *et al*., 2005; Sangwan *et al.*, 2007).

Amnion is blunt dissected from the chorion under sterile conditions (Figure I.75 a-e). Separate membranes are cut into fragments of different sizes and positioned with the epithelial part on nitrocellulose paper strips (Sangwan *et al*.,2007; Maral *et al*.,1999).

Mechanism of AM effects in ocular surface reconstruction

The AM grafts are used for reepitheliazation in cases of cornea burns, stromal ulceration and other defects (Lee *et al.*, 1997; Kruse *et al.*, 1999; Chen *et al*., 2000; Șapte *et al*., 2017).

The main important effects of the AM transplantation are: (1) facilitation of epithelial cell migration (Meller and Tseng, 1999; Meller *et al.*, 2002); (2) reinforcement of basal epithelial cell adhesion (Terranova and Lyall, 1986; Keene *et al.*, 1987; Sonnenberg *et al*., 1991); (3) promotion of epithelial cell differentiation (Guo and Grinnell, 1989; Streuli *et al.*, 1991; Kurpakus *et al.*, 1992); and (4) prevention of apoptosis (Boudreau *et al*., 1995; Boudreau *et al.*, 1996). The basement side of the AM is an ideal substrate for supporting the growth of epithelial progenitor cells by prolonging their life span and maintaining their clonogenicity (Tseng *et al*., 2001).

The stromal part of the AM includes a unique matrix component which suppresses TGF-β, the proliferation and differentiation of myofibroblasts and limbal fibroblasts of normal human corneal tissue (Tseng *et al.*, 1999; Tseng, 2001) and of normal conjunctival fibroblasts and pterygium body fibroblasts (Tseng, 2001; Lee *et al.*, 2000).

This mechanism explains why an AM graft reduce scars during the reconstruction of the conjunctival defect (Figure I.75,a-e) (Tseng *et al.*, 1997; Azuara-Blanco *et al.*, 1999; Tseng, 2001; Șapte *et al*., 2017), prevent recurrent scarring after pterygium removal (Prabhasawat *et al*., 1997; Kim *et al*., 1997; Shimazaki *et al.*, 1998; Ma *et al*., 2000; Solomon *et al.*, 2001) and reduce corneal haze, following phototherapeutic and photorefractive keratectomy (Choi *et al*., 1998; Park and Tseng 2000).

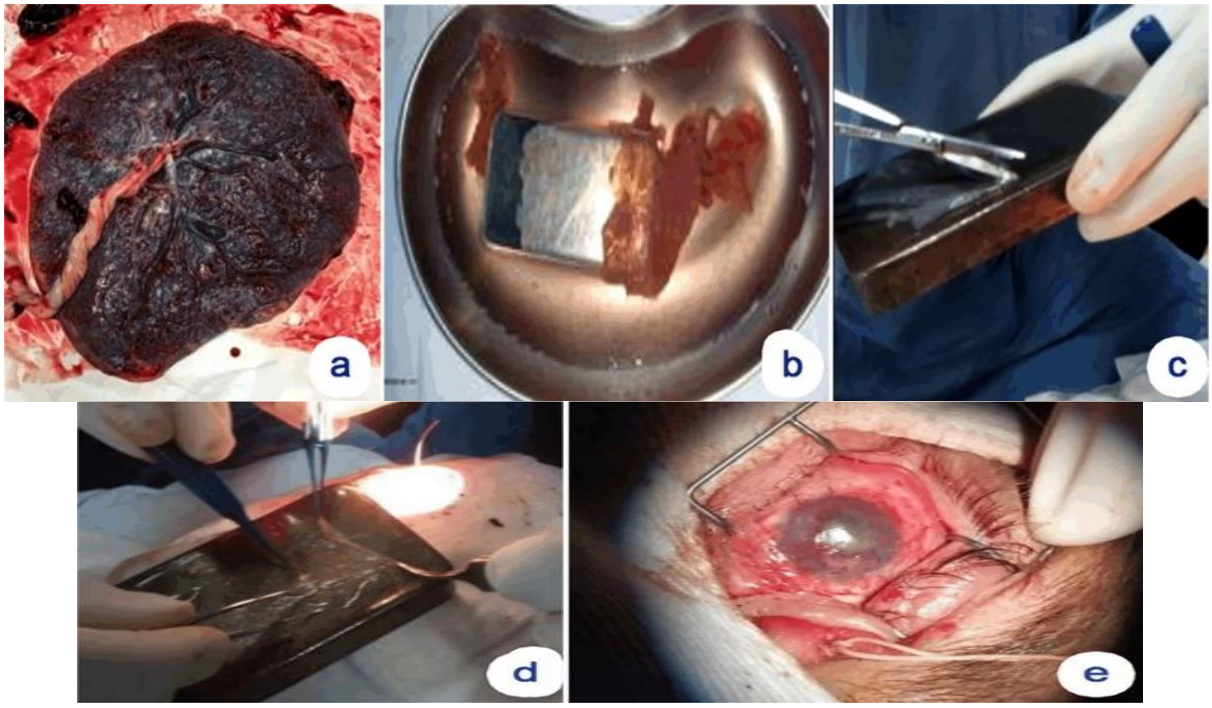

Figure I.75 – The preparation of fresh human AM: (a) AM is the inner most layer of the placenta; (b–d) The AM is trimmed to fit the size of the underlying defect of the bulbar conjunctiva; (e) AM is sutured to the bulbar conjunctiva of the patient (Șapte *et al*., 2017).

The anti-inflammatory effects properties of the AM grafts are determined by the inhibition of the expression of cytokines from the damaged ocular surface, e.g., interleukin (IL)-1α, IL-2, IL-8 are key regulators of the inflammatory response (Barnea *et al*., 2015), interferon-γ, necrosis factor-β, basic fibroblast growth factor, tumor and platelet derived growth factor (Solomon *et al.,* 2001). Shimmura *et al.,* in 2001, had demonstrated that the inflammatory cells were trapped and underwent apoptosis in the AM matrix (Shimmura *et al.,* 2001). Another important characteristic of AM is the production of several potent anti-angiogenic chemicals including endostatin, hrombospondin-1, and all four tissue inhibitors of metalloproteases (TIMP-1, -2, -3 and -4) (Hao *et al.*, 2000). Antibacterial effects of this valuable tissue have been showed against both Staphylococcus aureus and Streptococcus (Gram-positive cocci) as well as Pseudomonas aeruginosa and Escherichia coli (Gram-negative bacilli) (Kjaergaard *et al*., 1999; Kjaergaard *et al*., 2001), due to some antimicrobial factors in the amniotic fluid including bactricidin, lysozymes, beta-lysin, transferrin, and 7S immunoglobulin (Gusdon, 1962; Galask and Snyder, 1970). AM has another important characteristic, it shows lack of expression of the major histocompatibility antigens HLA-A, -B, or DR antigens (Akle *et al.*, 1981; Adinolfi *et al*., 1992), which allows for AM grafts to be transplanted on the eye surface (Șapte *et al*., 2017).

Clinical ophthalmic indications of the AM - The AM has another important property, it inhibits the formation of scars in the subconjunctival tissue, stimulating the growth and differentiation of conjunctival epithelial cells (Vanathi, 2012) and therefore it is considered to be a favorable and valuable substrate for eye surface reconstruction (Tsubota *et al.*, 1996; Tseng *et al*., 1998; Honavar *et al.*, 2000; Tsai *et al.*, 2000). Preserved or fresh hAM is used in the treatment of persistent epithelial defects in cases of autoimmune diseases, epithelial corneal defects, corneal ulcer, and infectious keratitis (Vanathi, 2012). For the diseases of the eye surface it is suppressing the acute or chronic host tissue inflammation and it is promoting healing with minimal scarring. AM can be used as a bandage applied on the surface of the corneal defect, or as a patch covering both the tissue and the damage site, in favour of the healing of underneath the host epithelium (Malhotra and Jain, 2014; Șapte *et al*., 2017). The use of AM as graft for eye conjunctiva surface reconstruction is preferred by ophthalmologists, in order to restore normal stroma and provide a healthy basement membrane for renewed epithelial differentiation and proliferation (Tseng, 2001; Șapte *et al*., 2017). In addition to the clinical indications for AMT mentioned by Bouchard and John (2004) and that could be found in Table I.7 (Bouchard and John, 2004). When a limbal stem cell deficiency is diagnosed, AMT is used in the management of the damaged limbal tissue, and it is aimed to restore the limbal stem cells (Vanathi, 2012).

Advantages and disadvantages of hAM grafting

One of the important roles of hAM is the reinforcement of the adhesion of basal epithelial cells and the facilitation of the epithelial cell migration, due to the thick structure of the hAM basement membrane (Vanathi, 2012).

Table I.7 – Indications of AMT (according to Bouchard and John, 2004)

AMT in the presence of stem cell deficiency	Chemical ocular injuries
AMT in the absence of stem cell deficiency	Epithelial cornea defects Corneal/sclera-corneal ulcers Bullous keratopathy
AMT for conjunctiva reconstruction	Pterygium Conjunctival chalasis OSSN Limbal dermoid Symblepharon Conjunctiva lesions Leaking blebs
AMT in ocular cicatricial diseases	Toxic epidermal necrolysis Ocular cicatricial pemphigoid Oculopalpebral and reconstructive surgery
Other indications of AM use	Stem cell cultures

The integration of the hAM graft was shown in different histological samples (Resch *et al.*, 2006). The integration of the hAM graft can be superficial, intraepithelial, subepithelial or intrastromal. This mechanism favors less vascularization in the healing process, and it maintains the transparency of the cornea, preserving the visual acuity (Vanathi, 2012).The hAM can not restore the eye surface in the situations of conjunctival epithelial stem cells loss and total loss of limbal epithelial stem cells (Tsubota *et al*., 1999).

Conclusions

Fresh or preserved hAM is an extremely valuable, inexpensive tissue used in eye reconstruction to date. The similarity of the histological structure of the hAM with that of the ocular structures (cornea, ocular conjunctiva) allows its use to supplement the defects occurring after ocular surface diseases. The procurement, processing and preservation of the hAM is easy and inexpensive, with multiple indications in ophthalmic surgery from pterygium to conjunctival or corneal neoplasia. Its use at the level of the ocular surface allows to obtain very good cosmetic results and contributes to the improvement of the patients' vision (Șapte *et al*., 2017).

I.4.2.2. Researches regarding chemical properties of human amniotic membrane for eye surface transplantation

Experimental part

HAM fragments were collected in septic conditions from donor mothers, who were operated on by caesarean section, tested serologically during pregnancy for communicable infectious diseases such as hepatitis B, C, HIV and syphilis. To determine the chemical composition, 7 fresh amniotic membrane fragments were processed to obtain a homogeneous cell, which was filtered and the supernatant centrifuged for 10 min (3500 rpm) (Costea *et al.*, 2018b). The concentration of glucose in the hAM was determined by the enzymatic method of glucose oxidase and the total proteins by the copper salt coupling method. The ionogram was determined using the Diestro 103 AP electrolyte analyzer (direct ISE potentiometry) (Costea *et al*., 2018). Total antioxidant capacity (TAC) was determined using the ABTS® colorimetric reagent method (2, 2'-Azinodi- [3-ethylbenzthiazoline sulphonate]) (Costea *et al.*, 2018b)

Results and discussions

Garby (1957) showed that there are similarities between the composition of hAM, amniotic fluid and plasma, with the same concentrations of sodium, potassium, chloride, creatinine and glucose (Garby *et al*.,1957). At birth the amniotic fluid has a glucose content of 23.4 ± 1.27 mg / 100mL, as compared to 86.03 ± 2.18mg / 100mL in the mother’s blood

and 63.5 ± 3.14 mg / 100mL in the fetus 'blood (Sozanskii,1961).The values we found of mean glucose concentration determined in the chemically analyzed hAM specimens (3mg / 100mL) were comparable to that in tears, reported by Balasubramanyam (Balasubramanyam *et al*., 2016) and those reported by Giardini and Roberts as 6.06mg / 100m\L (Giardini *et al*., 1950), (Tables I.8 and I.9).

Compared to serum values, the values found in the hAM were 20 times lower (Estridge *et al*.,2000). Schmidt *et al*., (1992) found similar values for glucose concentration in amniotic fluid and hAM composition (5-20 mg / 100 mL) (Schmidt,1992). The values obtained by us were similar (Costea *et al.*, 2018b) with those reported by Assali *et al*., (1972), (Assali *et al.*,1972).

The total protein concentration found by us in the amniotic membranes (0.07g / 100mL) (Tables I.8 and I.9) is 100 times lower than that in the serum (6-8g / 100mL), but comparable to that of the aqueous humor (0.004 mg / 100mL) (Costea *et al*., 2018b), as reported by Albert *et al.* (2008) (Albert *et al.*,2008). Some other authors noted that amniotic fluid proteins are principally of maternal origin, but have lower concentrations than in maternal serum (Jauniaux *et al.*,1991). Venkata *et al*., (2009) obtained a total protein value in tears in healthy individuals of 0.12 +/- 0.0479 / 100 mL (Venkata *et al*.,2009), which are similar to that of the amniotic membrane composition found by us (Costea *et al*., 2018b).

In 2018, Johnson in his study found lower protein values (0.026 g / 100mL) than we found (Jonhnson, 2018; Costea *et al.*, 2018b).

Table I.8 - Comparison Between Chemical Composition of hAM and Serum (Costea *et al*., 2018b)

Chemical composition of hAM	Authors			**Serum** (Estridge, Reynolds & Walters, 2000) [37]
	Costea *et al.*, 2018	Schmidt [38] 1992	Assali *et al.* [39] 1972	
Glucose (mg/100ml)	3	5-20	29.8	70-110
Total Proteins (g/100ml)	0.07	0.28-0.78	2.5	6.0-8.0
Na^+ (mEq/L)	155.4	-	133	135-148
K^+ (mEq/L)	5.74	-	4.9	3.5-5.4
Cl^- (mEq/L)	120.3	-	102.0	98-108
TAC (mmol/L)	1,1±0.1	-	-	-
pH	7.2	-	-	7.35-7.45

Table I.9 - Comparison Between Chemical Composition of hAM, Tears, and Serum (Costea *et al*., 2018b)

Chemical substance	Human Amniotic Membrane	Tears			Serum
	Costea *et al.* 2018	Balasubramanyam [35] 2016	Venkata *et al.* [42] 2009	Berman [45] 1991	Estridge, Reynolds& Walters 2000 [37]
Glucose (mg/100ml)	3	3-10	-	-	70-110
Total proteins (g/100ml)	0.07	0.6-2	0.12± 0.047	-	6.0-8.0
Na^+ (mEq/L)	155.4	142	-	120-165	135-148
K^+ (mEq/L)	5.74	15-30	-	20-42	3.5-5.4
Cl^- (mEq/L)	120.3	120-135	-	118-135	98-108
TAC (mmol/L)	1.1±0.1	-	0.7 ± 0.18	-	-
pH	7.2	-	7.4	-	7.35-7.45

Some authors have analyzed tear biochemistry by showing that the total protein concentration (0.5-2.0g/ 100mL) is lower than in serum (6-8g / 100mL), (Balasubramanyam, 2016; Sozanskii,1961; Gerard and Josset, 2011) yet the reported values are 10 times higher than those we obtained in the homogenate and hAM fluid (Costea *et al*., 2018b). The chemical tests run by us on the fresh hAM specimens revealed a slightly higher Na+ , K+ and Cl-, ion concentration (Na+ =152 mEq/L; K+=5.74 mEq/L; Cl- =131.6 mEq/L) than in serum (Garby,1957; Costea *et al.*, 2018b), yet comparable to that of ions in the aqueous humor(Na+=152 mEq/L; K+=3.9 mEq/L; Cl- =131.6mEq/L), as reported by Schrage (Schrage, 2011). Analyzing the composition of tears, Balasubramanyam reported higher values of Na +, K + and Cl- ions than in serum (Balasubramanyam,2016) but similar to those obtained by us from hAM fragments (Costea *et al*., 2018b). The pH value obtained in our study was 7.2, comparable to tear secretion (Costea *et al.*, 2018), reported by others authors to be 6.5 to 7.6 (Abelson *et al*.,1981).

We found TAC in hAM of 1.1±0.1 mmol/L (Tables I.8 and I.9) (Costea *et al.*, 2018) lower than that in serum (Schrage, 2011). However, a team of authors from India reported that tears from healthy people have a TAC of 0.7 ± 0.18 mmol/L, with a range of 0.41-1.03mmol/L (Venkata *et al*., 2009), i.e. similar values with those obtained by us for hAM (Costea *et al.*, 2018b).

Conclusions

The chemical composition of the hAM studied by us is similar to those reported by other authors in the medical literature. The data we found showed that the hAM has a chemical composition similar to that of tears and aqueous humor, but different from that of

serum, which recommends it as an extremely useful tissue in eye transplantation (Costea *et al*.,2018b).

I.4.2.3. Nano-scale modifications of human amniotic membrane induced by UV and antibiotic treatment: histological, AFM and FTIR spectroscopy evidence

Our research aimed is to investigate how the concentration of the antibiotic affected the structural properties of the hAM prepared as a graft for corneal reconstruction.

We also investigated the ultrastructural changes of hAM under UV and / or antibiotic treatment by combining histological/immunohistochemical examination with high-sensitivity atomic force microscopy (AFM) measurements and Fourier transform infrared (FTIR) spectroscopy.

Moreover, an *in vitro* enzymatic assay (collagenase digestion) was performed, along with a clinical case presentation (pterygium) requiring ocular surgery and reconstructing it with hAM graft, showing the most important outcome and advantages of this technique (Cavalu *et al*.,2021).

Materials and Methods

Procurement and preparation of biological tissue **-** The research protocol was performed in agreement with the ethical standards of the Helsinki Declaration and approved by the Ethical Committee of the University of Oradea, Romania (ref.no. 06 / 15.10.2020).

The biological tissue was obtained under strict aseptic conditions, from a patient who had undergone caesarean section at full term, with informed consent. Under a laminar flow hood, the membrane was washed with sterile physiological saline to remove blood clots, separated from the chorion by blunt dissection and peeled. Then, the hAM was cut into small-size (5 cm × 5 cm) specimens by using a sterile scalpel; each piece was again washed three times with sterile distilled water and flattened on individual Petri dishes, to which 5 mL PBS (Phosphate Buffer Saline) was added and stored at −20 ° C until further treatments and preparation for histological examination, FTIR and AFM measurements (Cavalu *et al*.,2021).

Antibiotic and UV Treatment **-** The hAM specimens were divided into 4 groups and the following treatments were applied:

i. Antibiotic treatment: Specimens were allowed to interact with the gentamicin injectable solution (KRKA, Novo Mesto, Slovenia) concentrations of 40 and 80 mg/mL, for 1 h, and then they were washed with PBS, rinsed with sterile distilled water, flattened on a cellulose support and stored in a refrigerator until FTIR and AFM investigations. The samples were labelled AG40 and AG80, respectively, according to each concentration.

ii. UV treatment: Specimens were exposed to UV in air using a GL4 germicidal lamp

(Philips TUV 6W G6) at no more than 254 nm, for 1 h, and then kept in a refrigerator until further investigations. The samples were labelled AUV.

iii. Combined antibiotic/UV treatment: Immediately after gentamicin treatment (concentration 40 mg/mL), specimens were exposed to the UV treatment described above and then kept in a refrigerator until further investigations. The samples were labelled AGUV.

iv. The control sample was the natural amniotic membrane without any treatment, labelled AMN.

The above treatments were carried out in triplicate (Cavalu *et al*.,2021).These specimens were examined histologically and immunohistochemically, and also analysed spectroscopically (Cavalu *et al*.,2021).

The percentage of protein secondary structures (α-helix, β-sheets, turns, random and side chains) was calculated based on the area under each peak, and the assignments of the components were conducted according to the literature (Cavalu *et al*.,2015; Bridelli,2017; Belbachir *et al*.,2009).

Results were represented as mean ± standard deviation (SD) for three independent experiments. A probability level of $p < 0.05$ was considered statistically significant, and the analysis was conducted by Student's t-test.

Atomic force microscopy (AFM) measurements were used to obtain the ultrastructural details of the amniotic membranes, air-dried, after different treatments.

The thickness of the individual collagen fibers were measured, recording their profile (Cavalu *et al.*,2021).

Enzymatic (Collagenase) Degradation Assay **-** The hAM specimens prepared were allowed to dry, and then they were weighted (balance model FA-G Want Balance Instr. Changzhou, China), immersed individually in a 0.1% collagenase solution (Sigma–Aldrich. St. Louis, MO, USA) with pH = 7 and incubated at 37 °C (incubator Model MCO-5 AC, Sanyo/Panasonic Biomedical, York, UK) in static conditions, under a flux of 5% CO_2. After different time intervals (12, 24, 36, 48, 60 and 72 h), the specimens were carefully removed and allowed to dry completely and weighted again.

The weight of the remaining mass was expressed as a percentage (mean value ± SD) and the statistical significance was measured by an ANOVA test. A p-value of less than 0.05 was considered significant (Cavalu *et al.*,2021).

Clinical Case **-** A case of recurrent pterygium was presented, in which fresh hAM was successfully applied in order to reconstruct the corneal and bulbar conjunctiva surface, after the surgical removal of the grade 3 recurrent pterygium.

The patient (30 years old, male, living in a rural area) was previously informed about the surgical procedure and he gave his informed consent for inclusion in the study, in accordance with the Declaration of Helsinki and the research protocol approved by the Ethical Committee of the University of Oradea, Romania (ref. nr. 06/15.10.2020).

Before surgery, a complete ophthalmologic examination including measurement visual acuity, intraocular pressure, and biomicroscopy were performed (Cavalu *et al*.,2021).

Statistics **-** Results were expressed as mean ± standard deviation for three independent experiments, using Student's t-test in the case of FTIR measurements, while one way analysis of variance (ANOVA) was employed for the enzymatic digestion assay. In both cases, significance was accepted with $p < 0.05$ (Cavalu *et al*.,2021).

Results

Histological Examination **-** The details of HE staining revealed a uniform layer of cubic cells displayed on a basement membrane of the hAM sample (Figure I.76 a), while the expression of collagen IV in the basement membrane is continuous and dense (Figure I.76 b).

After exposure to UV light, a moderate loss of cubic cells can be seen in several spots (Figure I. 76 c), with a dense and continuous expression of collagen IV; a splitting of about 8 μm in collagen fibrils was detected in a single spot (Figure I.76 d).

The cuboidal cells slightly changed their size and shape after the treatment.

The loss of cubic cells can be noticed also after gentamicin treatment (Figure I.76 e), accompanied by a splitting of about 3 μm in collagen fibrils, over a relatively long distance (Figure I.76 f). By applying a double gentamicin concentration, the basement membrane maintained its integrity, but the loss of cubic cells can be noticed in a higher number of foci (Figure I.76 g), while the splitting of the collagen fibrils is more significant, the distance between the two expressions being variable, from 8 to 20 μm (Figure I.76 h).

The UV exposure of gentamicin-treated specimens showed extensive loss of cubic cells (Figure I.76 i), concomitantly with a significant splitting of collagen fibrils (about 70 μm) over a long length, as shown by the expression of collagen IV (Figure I.76 j) (Cavalu *et al*.,2021).

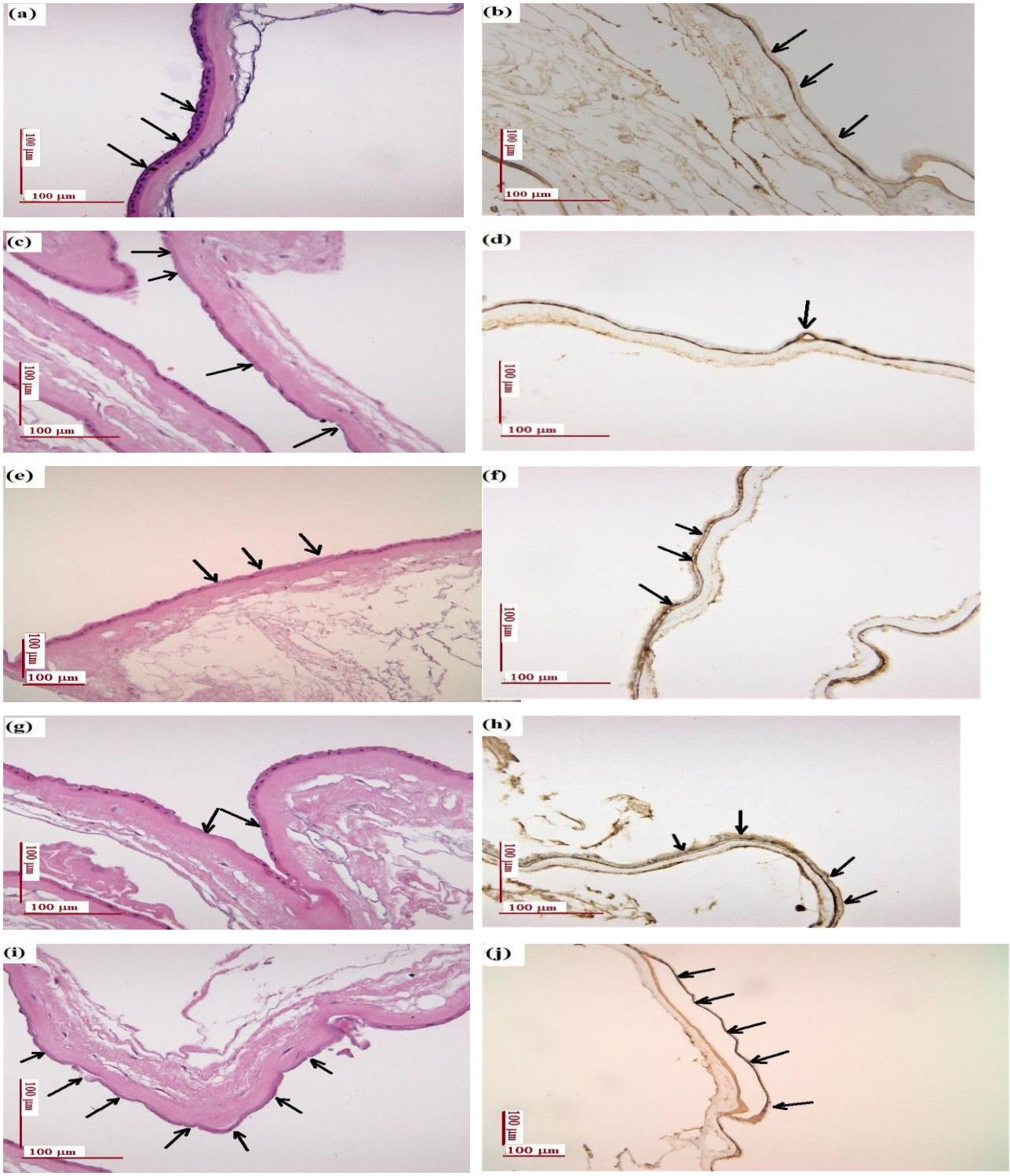

Figure I.76 - Histological and immunohistochemical examination: (a,b) natural amniotic membrane (AMN); (c,d) amniotic membrane exposed to UV for 1 h (AUV); (e,f) amniotic membrane treated with gentamicin (40 mg/mL) (AG40); (g,h) amniotic membrane treated with gentamicin 80 mg/mL (AG0); (i,j) amniotic membrane treated with gentamicin 40 mg/Ml and exposed to UV for 1 h (AGUV). Left panel: H&E staining; right panel: immunohistochemistry staining of collagen IV (antibody clone CIV 22). Scale: 100 µm (Cavalu *et al*.,2021).

FTIR Spectroscopy

The FTIR spectra recorded in the range 400–4000 cm−1 are presented in Figure I.77, a-e for AM specimens belonging to each treatment group, while in Figure I.78, the FTIR spectrum of pure gentamicin is presented.

Under the action of UV and / or gentamicin, there is a structural alteration of the proteins.

A decrease in α-helix and β-sheet content, accompanied by increased content in less ordered structures (turns, random and side chains) was noticed after all treatment.

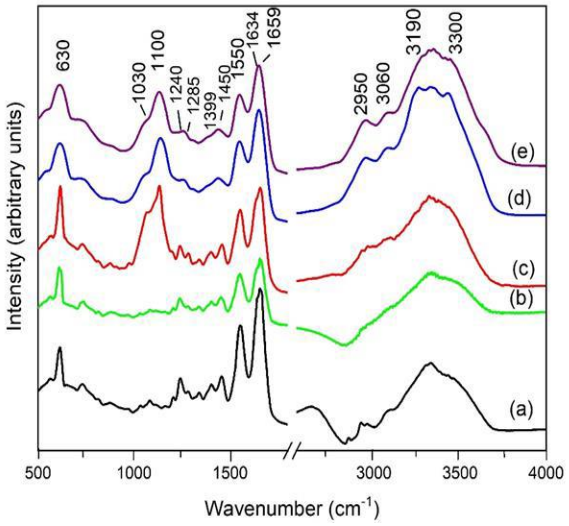

Figure I.77 - FTIR spectra of AM specimens after treatment with antibiotic and/or UV: (a) natural AM without any treatment (AMN); (b) AM exposed to UV for 1 h (AUV); (c) AM treated with gentamicin (40 mg/mL) and exposed to UV for 1 h (AGUV); (d) AM treated with gentamicin 40 mg/mL (AG40); (e) AM treated with gentamicin 80 mg/mL (AG80) (Cavalu *et al*.,2021).

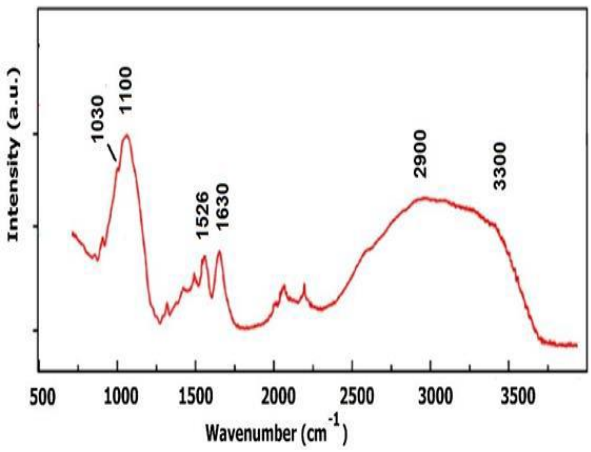

Figure I.78 - FTIR spectrum of gentamicin (Cavalu *et al*.,2021).

Nanotopography: AFM Examination

The 3D and 2D topographic aspects of the hAM after the different treatments presented in Figure I.79, a-j, indicate the details of a single collagen fibril exposed on the surface (W = width; H = height) (Cavalu *et al*.,2021).

The application of gentamicin causes an increase in the width of the collagen fibril (Cavalu *et al*.,2021).

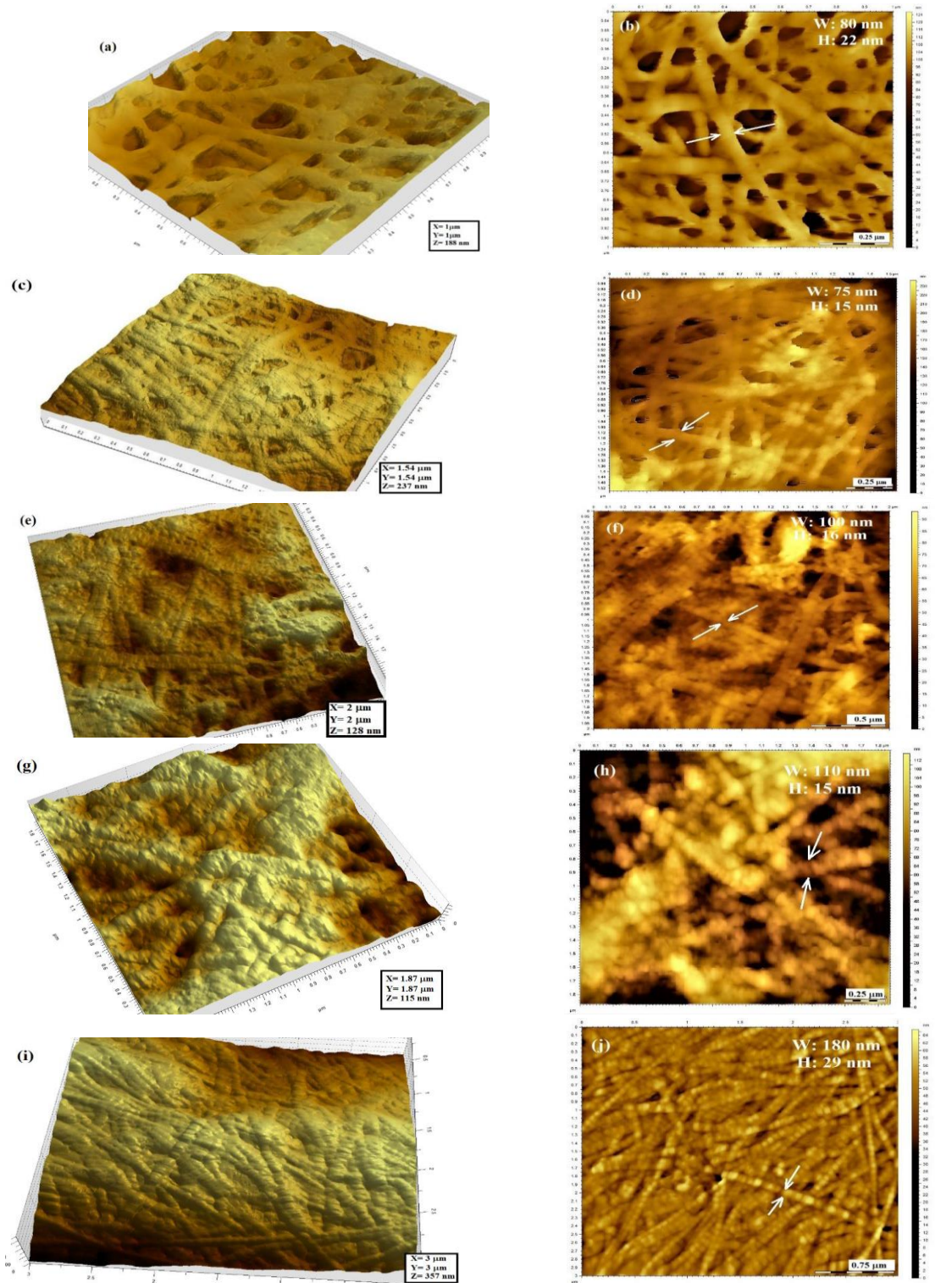

Figure I.79 - AFM examination of the amniotic membrane after different treatments (3D and 2D images) indicating the features of a single collagen fibril: (a,b) natural membrane (no treatment) (AMN); (c,d) membrane exposed to UV (AUV); (e,f) membrane exposed to gentamicin treatment 40 mg/mL (AG40); (g,h) membrane exposed to gentamicin treatment 80 mg/mL (AG80); (i,j) membrane exposed to gentamicin and UV treatment (AGUV). The profiles of a single collagen fibril exposed on the surface of the amniotic membrane after different treatments are presented in the Supplementary Materials (Figure S1) (Cavalu *et al.*,2021).

Collagenase Digestion Assay

Figure I.80 presents the results of the collagenase digestion of the hAM prior to any treatment and following the UV and/or gentamicin treatment monitored for 72 h.

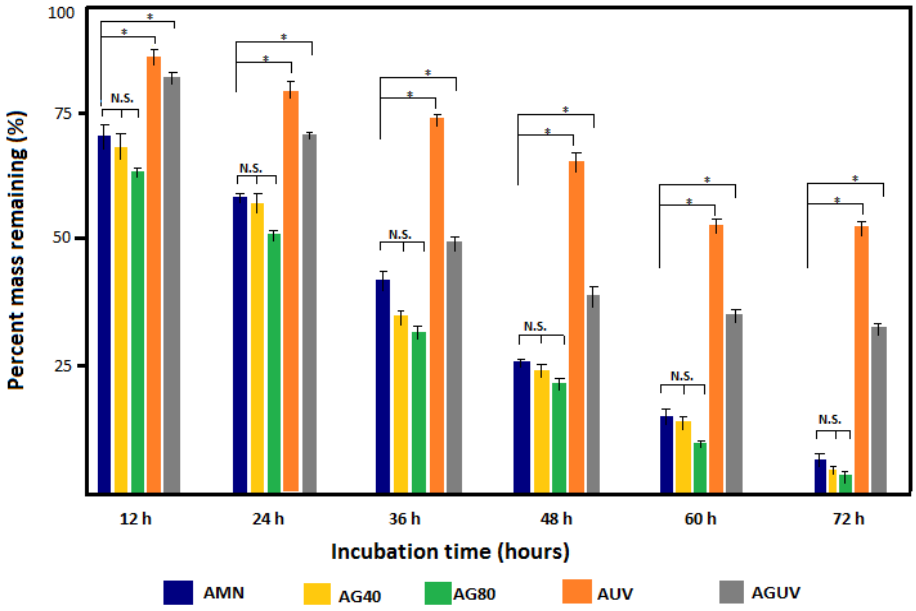

Figure I.80 - Collagenase digestion of the amniotic membrane after different treatments: natural membrane (AMN), gentamicin treatment with 40 mg/mL (AG40), gentamicin treatment with 80 mg/mL (AG80), 1 h exposure to UV (AUV) and 1 h exposure to UV after gentamicin treatment (AGUV). $p < 0.05$ was considered significant; N.S.—non-significant (Cavalu et al.,2021).

Within the first 12 h, a drastic degradation of AMN, AG40 and AG80 specimens was noticed, compared to AUV and AGUV specimens, and this behavior had a similar, continuous trend for the entire time interval.

At each time point, insignificant modifications were noticed between gentamicin-treated samples and untreated ones, no matter the antibiotic concentration. After 72 h of digestion, less than 8% of AMN, AG40 and AG80 remained undigested, while the UV-treated specimens (AUV and AGUV) showed enzymatic resistance of 55% and 32%, respectively, in terms of the mass remaining.

By comparing both UV-treated specimens, it can be noticed that gentamicin strongly influences the degradation behavior, which is reflected in the remaining mass when comparing AUV and AGUV percentages at each time point. The difference is more obvious in the time interval 36–72 h. However, for the last 12 h, a slower degradation of AUV and AGUV specimens was noticed (Cavalu *et al*.,2021).

Clinical Case

Figures I.81, a–e present a clinical situation in which the hAM was used in ophthalmologic surgery, as a grafting biomaterial, to cover the remaining tissue defect due to the pterygium excision (or its recurrence).

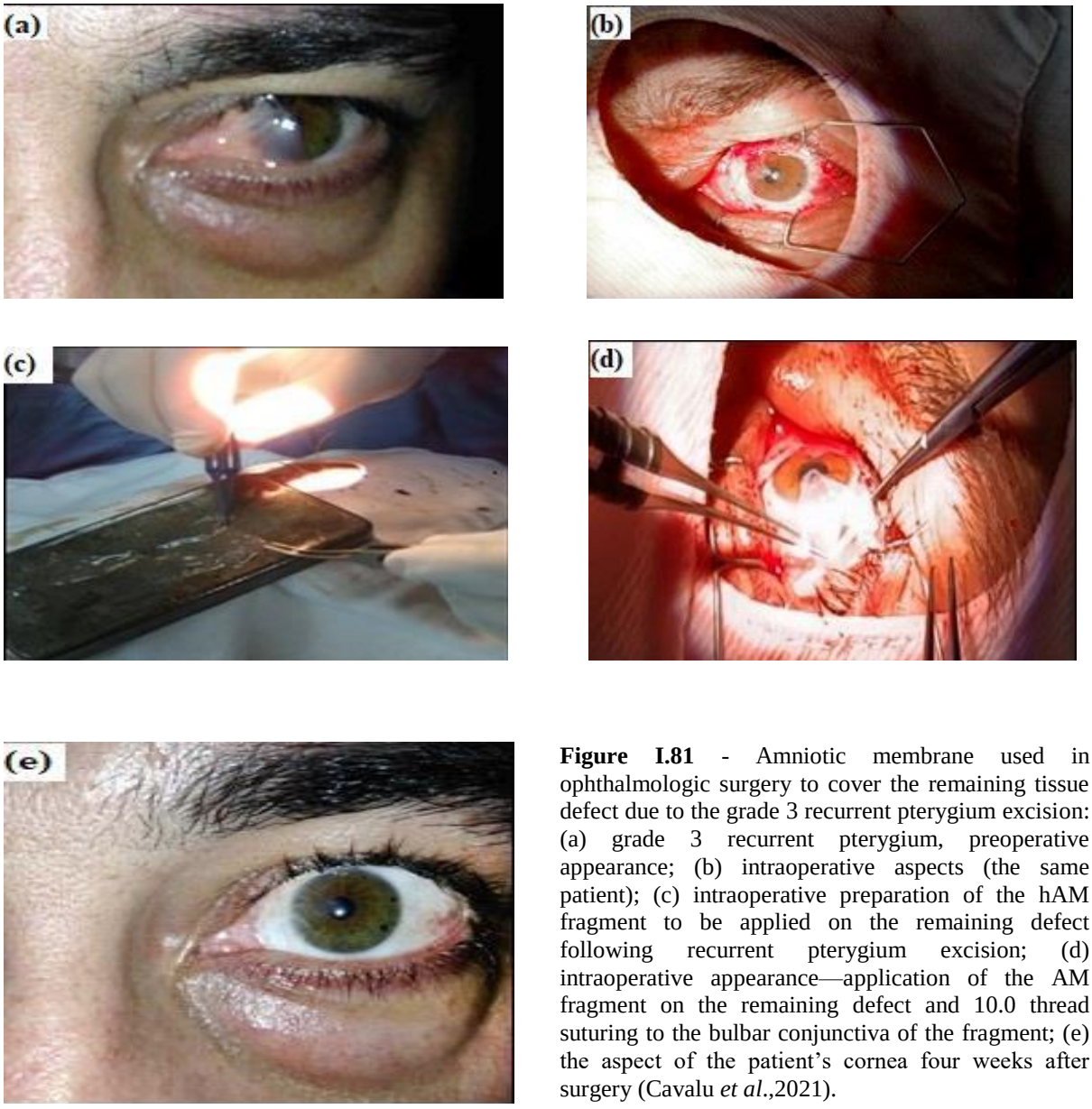

Figure I.81 - Amniotic membrane used in ophthalmologic surgery to cover the remaining tissue defect due to the grade 3 recurrent pterygium excision: (a) grade 3 recurrent pterygium, preoperative appearance; (b) intraoperative aspects (the same patient); (c) intraoperative preparation of the hAM fragment to be applied on the remaining defect following recurrent pterygium excision; (d) intraoperative appearance—application of the AM fragment on the remaining defect and 10.0 thread suturing to the bulbar conjunctiva of the fragment; (e) the aspect of the patient's cornea four weeks after surgery (Cavalu *et al*.,2021).

The standard protocol during pterygium surgery was applied, involving the peeling of the abnormal fibro-conjunctival tissue, on the surface of the cornea by blunt dissection of the pterygium head. The excision of adjacent residual pterygium tissue was also performed, along with the fibrovascular tissue. The hAM graft was cut to fit over the conjunctival and corneal defect, with the stromal side down, in order to promote epithelial adhesion and rapid healing. Immediately after surgery, the eye was patched. The post-surgical treatment

consisted of gentamicin sulfate 3%, dexamethasone and artificial tear eye instillations and a recommendation of total protection against UV radiation exposure. The patient was examinedfirst day and seven days after the surgery, and then after one month. After four weeks, complete healing of the corneal tissue was noticed (Cavalu *et al*.,2021).

Discussions

As described in the literature, there are three different surgical techniques: graft or inlay, patch or overlay and combined multilayer techniques (Cauchi *et al*.,2008). In the graft or inlay technique, the hAM is administered as a permanent basement membrane substitute, being integrated in the host tissue and acting as a scaffold for epithelial cells to grow. This technique (graft or patch) is usually applied to persistent epithelial defects, corneal ulceration or conjunctival tumors. In the patch or overlay technique, the hAM is placed temporarily on the ocular surface, acting as biological bandage, but not being integrated into the host. In this case, the epithelium is expected to grow underneath rather than over the top, and the hAM will disassociate from the ocular surface after a certain time (Krysik *et al*.,2020). Regardless the surgical technique employed, topical antibiotic application is required in order to eliminate the risk of infections. The overall goal of our study was to investigate the structural and morphological modifications of the collagen matrix in the AM, by the means of histological observation, FTIR spectroscopy and AFM images, upon treatment with gentamicin and exposure to UV-C radiation. In particular, we also aimed to investigate to what extent the antibiotic concentration will influence the structural properties of the hAM prepared for corneal reconstruction (Cavalu *et al*.,2021). After treatment with gentamicin, regardless of its concentration, there is a separation of collagen fibers that have the role of maintaining the integrity of the basement membrane. After UV treatment, a double-chain separation of collagen fibrils from the hAM structure is found. Major changes are seen after antibiotic treatment, followed by UV radiation for one hour; extended cuboidal cell loss is observed along the length of the basement membrane and the splitting of collagen fibrils was observed over a very long length, these being highlighted by immunohistochemical reactions. FTIR investigations revealed that as a result of the treatments applied, the structures of α-helix and β-sheet content underwent important changes (Cavalu *et al*.,2021). All the treatments applied in our study indicate a decrease in α-helix and β-sheet content, accompanied by increased content in less ordered structures, such as turns and random and side chains, while the position of the main FTIR fingerprints was slightly shifted toward higher wavelengths (Cavalu *et al*.,2021). The results are consistent with previously reported data (Rabotyagova *et al*.,2008), which evidenced the behavior of amide I band components in

both native and denatured forms of collagen due to UV treatment, suggesting a helix–coil transformation of collagen. The triple-helical regions of collagen are known to be stabilized by hydrogen bonding and van der Waals attractions between imine residues on different chains (Lai *et al*.,2014). Both chemical and UV crosslinking of collagen are associated with its denaturation due to the collapse of hydrogen bonds in polypeptides. Glutaraldehyde, chitosan, poly (2-hydroxyethyl methacrylate), riboflavin and carbodiimide were reported in the literature as efficient crosslinking agents for the hAM (Lai *et al.*,2013; Zhang *et al.*, 2020; Spoerl *et al.*,2004). On the other hand, the crosslinking process enhances the biostability of the AM matrices to support limbal epithelial cell growth and can significantly improve the mechanical properties of the collagen matrix (Zhang *et al*.,2020). Therefore, although the crosslinking may cause denaturation of the collagen matrix, *in vitro* experiments of cultures on modified AM samples demonstrated that epithelial progenitor cells were preserved more effectively and the benefits of crosslinking prevail in terms of AM functionality (Lai *et al.*,2014). Moreover, some previous reports demonstrated that ex vivo expansion of limbal epithelial cells occurs at a faster rate on the decellularized hAM (with sodium dodecyl sulphate) compared with the fresh, natural one (Figueiredo et al.,2017). However, according to some authors (Stancanelli *et al*.,2008; Spoerl *et al*.,2004), a lower sheet/turn ratio after the treatment indicates inferior biocompatibility when compared to the native hAM. Based on the quantitative results presented in Figure I.82, the sheet/turn ratio has an average value of 2.27 for the AMN specimens, 1.83 for AUV, 1.88 for AG40, 1.70 for AG80 and 0.83 for AGUV specimens.

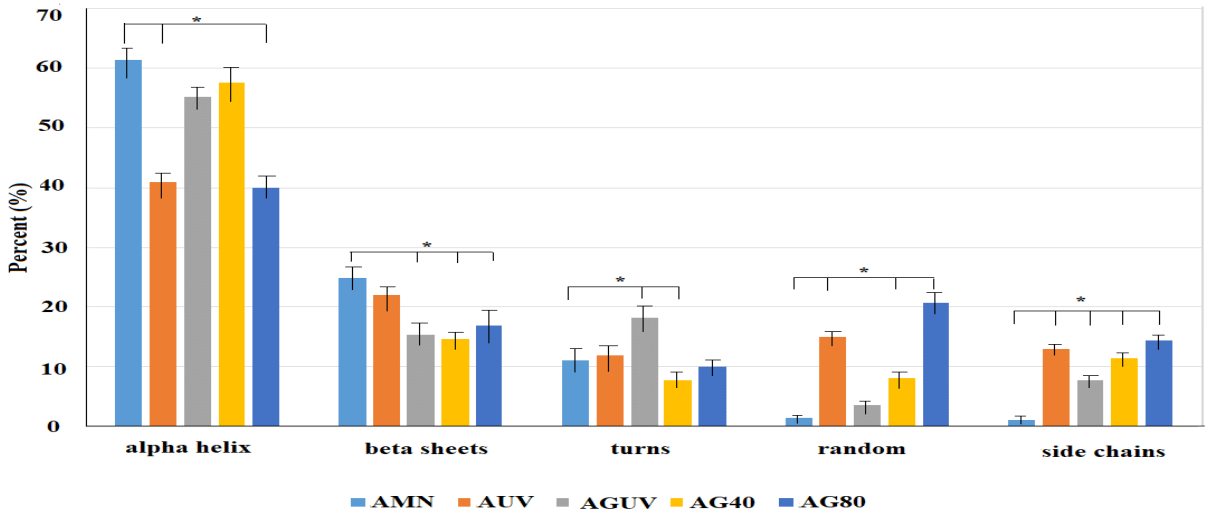

Figure I.82 - Quantitative analysis (percent) of the collagen secondary structure in the amniotic membrane after different treatments: natural membrane (AMN), 1 h exposure to UV (AUV), gentamicin treatment (40 mg/mL) and 1 h exposure to UV (AGUV), gentamicin treatment 40 mg/mL. * $p < 0.05$ was considered significant (Cavalu *et al*.,2021).

The lowest value (0.83) indicates the lowest biocompatibility, which was noticed for AGUV specimens, the results being also supported by the histological observations (Cavalu *et al*.,2021). Nanotopographic measurements on the surface of the hAM and after the application of various treatments, showed the details of collagen fibrils in the 3D network, which is influenced by antibiotic treatment and UV exposure (Cavalu *et al*.,2021). Stylianou *et al*. demonstrated that in the nanoscale range, the collagen topography and biological properties are affected by UV radiation, which may induce alterations in cell behavior (Stylianou *et al.*,2014). There are no other studies in the literature that show that gentamicin treatment has an influence on the ultrastructural changes of hAM. The collagenase digestion assay performed in our study demonstrated that UV exposure significantly reduces the degradation rate of AM, including the prior gentamicin treatment, while the gentamicin concentration has an insignificant influence on the dissolution time (Cavalu *et al*.,2021). It is well known that fresh amnion usually dissolves within 1 week (Spoerl *et al*.,2004), but the crosslinking procedures (by glutaraldehyde, carbodiimide, riboflavin, UV radiation) significantly increase resistance to collagenase digestion, especially in the anterior part of the cornea (Fujisato *et al*.,1999; Lai, 2014; Zhang *et al*.,2020; Spoerl *et al*.,2004). To highlight the clinical impact of our research, we presented a clinical case of recurrent grade 3 operated pterygium in which the remaining defect was supplemented by a fresh hAM graft. Complete healing occurred after 4 weeks with complete graft integration and excellent postoperative cosmetic appearance of the patient. Postoperative treatment consisted of instillations with gentamicin sulfate (3%) every 2 hours and protection against UV radiation (Cavalu *et al*.,2021).

Conclusions

Based on histological examination and complementary AFM and FTIR spectroscopy, we investigated the ultrastructural modification of the hAM upon gentamicin treatment and/or UV exposure, in the context of its suitability to be used as a graft in corneal reconstruction. The morphological features evidenced by HE stain and immunohistochemical investigations were correlated with structural and ultrastructural modifications. The loss of cuboidal cells in the basal membrane of the hAM was accompanied by the splitting of collagen fibrils, which was in concordance with the structural alteration of collagen molecules, as evidenced by the FTIR quantitative analysis of the protein's secondary structure. At the nano-scale, AFM details showed modifications of collagen fibrils in terms of their thickness and network compaction upon gentamicin and/or UV treatment. The collagenase digestion assay demonstrated that UV exposure significantly reduces the

degradation rate of the AM, while gentamicin treatment promotes an accelerated enzymatic digestion of the hAM upon UV exposure. Within the limitations of our study, the results might have importance in the context of the current debate over whether the hAM should be used intact (fresh or preserved at −20 °C) or photo-crosslinked as a grafting material, taking into account the denaturation influenced by post-surgical eye antibiotic treatment (Cavalu *et al*.,2021).

I.4.3. Researches regarding oculo-orbital traumas and the impact upon the patients

Background

Approximately 1.6 million people lose their visual acuity each year, most commonly affecting adults and the elderly (Glynn *et al.*, 1988; Desai *et al*., 1996; Mac Ewen, 1999; Nadeem *et al.*, 2013).

The most common causes of eye injuries are domestic accidents and one in twenty patients consult an ophthalmologist for these causes (Mac Ewen, 1999).

All the eye traumas are connected with eye loss and ther reconstruction of the cavity with ocular prosthesis for improuving the esthetic aspect of the patient and for his phychological well being. In the field of oculo-orbital traumatology, with psychological and forensic implications especially in aquired anophthalmia, I published the following articles:

Claudia Florida Costea, Anca Sava, Gabriela Florența Dumitrescu, Mircea Albert, Andrei Cucu, Șerban Turliuc, Dana Turliuc, Forensic Aspects of Ocular Trauma, *Aperito Journal of Ophthalmology*, 2015a, 1(2),109:1-5.

Costea CF, Turliuc D, Sava A, Dumitrescu GF, Cucu A, Turliuc S. Principles and guidelines involved in the management of surgical acquired anophthalmia patients, *Romanian Journal of Oral Rehabilitation*, 2016a, 8(1): 59-64.

Costea CF, Cucu AI, Dimitriu G, Brosteanu M, Turliuc S, Dumitrescu GF, Sava A, Turliuc DM. Understanding the psychological impact in a clinical case of eye globe rupture with forensic implications, *Romanian Journal of Oral Rehabilitation*, 2016b, 8(2): 61-67.

Turliuc DM, Cucu AI, Arbore-Sorete R, Dumitrescu GF, Sava A, **Costea CF.** Orbitocranial penetrating injury by a metallic foreign body. Case report and anatomical considerations. *Romanian Neurosurgery*, 2017a, 31(4):437-443.

Turliuc DM, Costan VV, Cucu AI, **Costea CF**. Intraorbital Foreign Body. *Revista Medico-chirurgicală a Societăţii de Medici şi Naturalişti din Iaşi*, 2015a, 119(1):179-184.

I.4.3.1. Forensic aspects of oculo-orbital trauma in adults

We conducted a retrospective study between 2010 and 2012 on 109 patients with eye injuries, hospitalized in the Second Ophthalmology Clinic of the "Prof.Dr.Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania. We studied the demographic and anatomical data of these patients, analyzing the age, sex, type of trauma and eye structures involved (Costea *et al.*,2015 a).

Results and Discussions

Of the 6839 patients hospitalized in the Second Ophthalmology Clinic Iași, Romania between 2010-2012, 1.59% suffered eye injuries (Figure I.83). More than half of the number of affected patients were male (76.14%); n = 83, the male / female ratio being 3.1) (Costea *et al.*,2015 a). Both eyeballs were affected in approximately the same percentage, 53.22% right eye and 46.78% left eye. Penetrating eye injuries (Table I.10) were most common in a number of 96 cases (88.0%) requiring eyeball reconstruction (Costea *et al.*,2015a). The most common eye injuries were penetrating corneal wounds and traumatic cataracts (Table I.11) (Costea *et al.*,2015a).

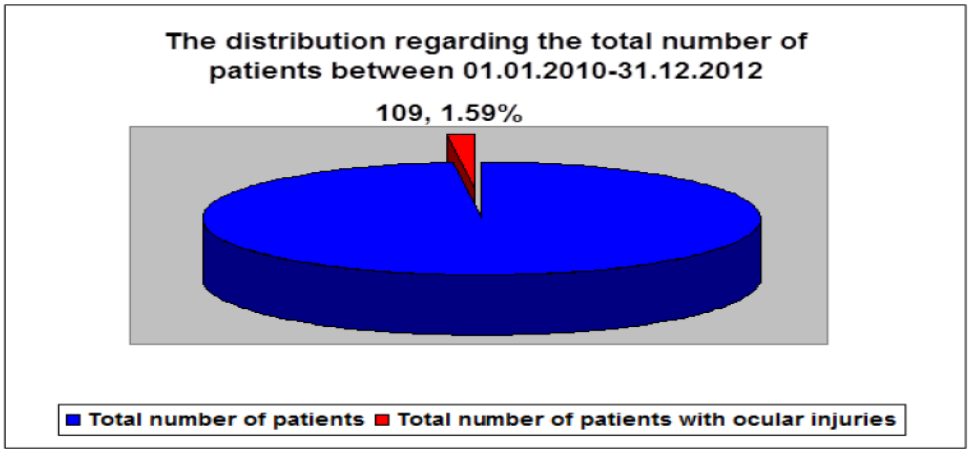

Figure I.83. Percentage of patients with eye injuries over a period of 3 years (Costea *et al.*, 2015a).

Table I.10. Types of eye injury according to the etiological mechanism (Costea *et al.*, 2015a)

Type of ocular trauma	Number of cases (%)
Penetrating/perforating trauma	96 (88.07)
Non-penetrating trauma	6 (5.50)
Penetrating/perforating trauma with intraocular foreign body	7 (6.43)

Table I.11. Patterns of eye injury according to the impaired eye structures (Costea *et al*., 2015a)

Structural damage to the eyeball	Number of cases
Eyelid wound	2
Conjunctival wound	11
Corneal abrasion /corneal ulcer	3
Corneal leucoma	13
Corneal siderosis	1
Corneal wound	39
Corneo-scleral wound with iris hernia	11
Corneal wound with iris hernia	12
Scleral wound	28
Traumatic cataract	48
Dislocation / subluxation of the lens	5
Irido-dialysis / iris coloboma	3
Traumatic exogenous uveitis	20
Hyphaema	3
Secondary Glaucoma	10
Vitreous bleeding	5
Retinal detachment	9
Macular edema	1
Hemophtalmus	14
Endophtalmitis	5
Traumatic optic neuropathy	1
Eyeball atrophy	3
Orbital cellulitis	2

In a similar study, Parman *et al.* found that the most affected eye structures were the cornea (47.60%), the iris (32.64%) and the eyelids (25%) (Parman *et al.,* 1985). In our study, the most affected was also the cornea (Costea *et al*., 2015a) data that are consistent with those published in the literature. Penetrating or non-penetrating trauma results in loss of the eyeball, which results in facial disfigurement or other post-traumatic sequelae such as entropion, ptosis, and strabismus with severe forensic implications (Sharma *et al.*, 2008). In these forensic cases the patient should be examined carefully and all lesions properly documented, in terms of visual acuity, eye reflexes and eye motility (Costea *et al.*,2015 a).

Other paraclinical examinations should also be considered: ocular ultrasound, CT and MRI orbital and craniocerebral scan (Pokhrel and Loftus, 2007) In Romania, the loss of the eyeball or visual acuity as a result of an aggression is punished by the Penal Code, with imprisonment between 2 and 10 years (Antoniu *et al*., 2011). Post-traumatic anophthalmia

has a devastating emotional effect on the patient, regardless of age and social status (Costea *et al.*,2016 b).

In these cases, the ophthalmologist, neurosurgeon and plastic surgeon must take into account the bioethical principles of Beauchamps and Childress (the principle of nonmaleficence anatomy, principle of justice and beneficence) and apply them in each case of acquired anophthalmia (Costea *et al*., 2016a).

Eye injuries are ophthalmic emergencies and require immediate treatment (Figure I.84, A-C), the patient being evaluated and examined in detail in order to provide the correct forensic evidence to the authorities (Costea *et al.*,2016a).

The mental impact is a serious consequence of patients diagnosed with severe eye trauma who eventually end up with prosthetic anophthalmia, often being diagnosed with acute post-traumatic stress, anxiety, depressive episodes and suicidal tendencies (Costea *et al*.,2016b).

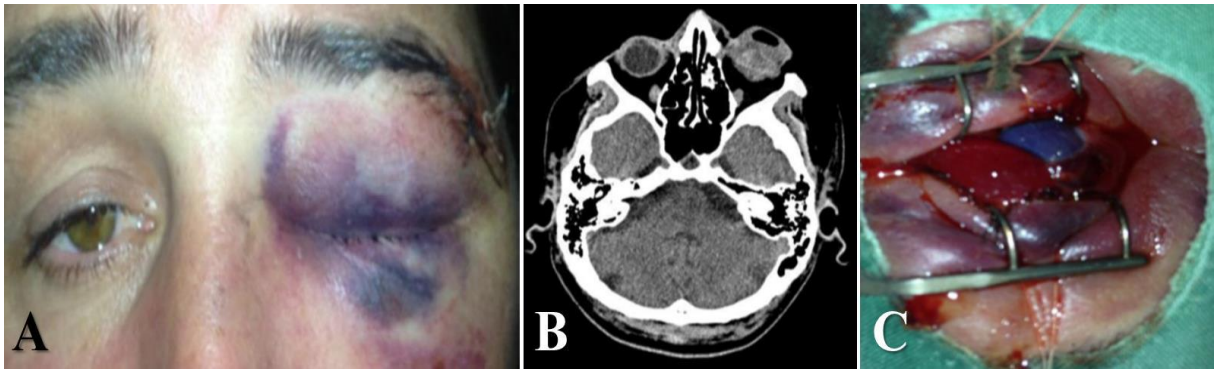

Figure I.84. Preoperative appearance: eyelid hematoma, traumatic ptosis and left eyebrow wound (A). Axial CT scan showing complete destruction of the left eyeball (B). Operating microscope view of an eyeball rupture secondary to blunt trauma with a metallic blunt object. Subconjunctival hemorrhage of this severity raised suspicion of eyeball rupture (C) (Costea *et al*., 2016a)

I.4.3.2. Orbito-cranial traumas in adults and children: case studies

Clinical Case No. 1

The 38-year-old male patient was admitted to the Neurosurgery Clinic with penetrating orbital-cranial trauma (OPI) with a foreign metal body during a work accident. At admission, the patient had GCS = 15, with headache and bradypsychia.

Ophthalmologic examination revealed a hematoma in the upper eyelid and a wound in the medial corner of the eyelid and visual acuity being "without light perception" in the right eye (Figure I.85 A and B). The blunt metal body was removed by the patient after the accident (Turliuc *et al*.,2017a).

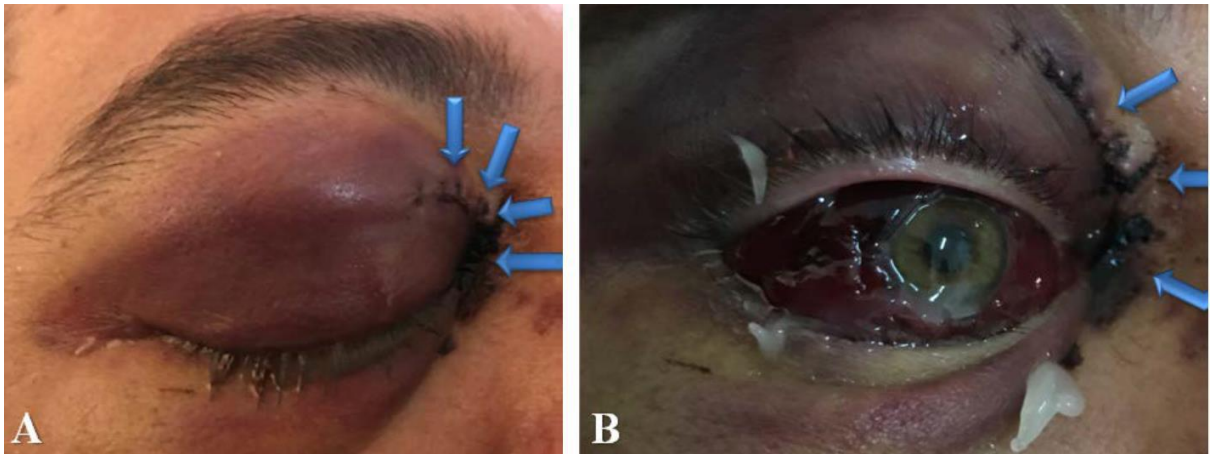

Figure I.85 - Orbital ecchymosis with sutured wound (blue arrows) (A). Right subconjunctival hemorrhage and the entry point of foreign body (blue arrows) (B) (Turliuc *et al.*, 2017a).

CT examination showed a trajectory of orbital ceiling fracture, right frontal laceration and frontal intracerebral hemorrhage (Figure I.86, A-C and I.87, A-E).

The clinical evolution of the patient was favorable, but with the loss of visual acuity in the right eye (Turliuc *et al.*, 2017a). Ocular ultrasound revealed vitreous hemorrhage, retinal detachment, and choroidal hematoma in the right eye (Figure I.87 C and F).

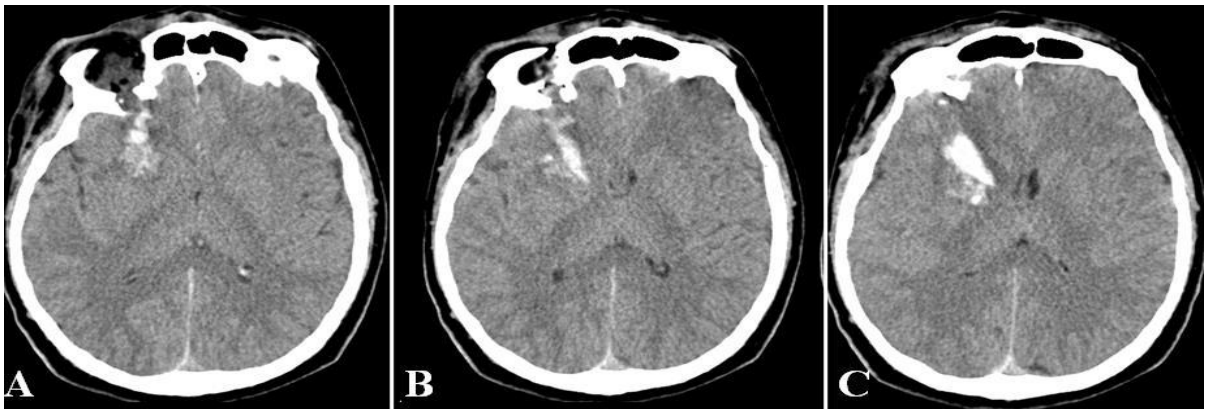

Figure I.86 - Head CT-scan with: right orbital roof fractures (A, B), right frontal dilaceration and intracerebral hemorrhage (B, C) along the trajectory of the foreign body (Turliuc *et al*., 2017a).

Depending on kinetic energy, OPIs are divided into (1) injuries with low-velocity and (2) injuries with higher-velocity (Mackerle and Gal, 2009, Mzimbiri *et al*., 2016).

In our patient's case, OPI had high-velocity, and this type of trauma produces fractures of the orbital walls and penetrates the intracranial space in a direction of foreign body trajectory (Lee *et al.*, 1999, O'Neill *et al.*, 1994, Scarfo *et al.*, 1990).

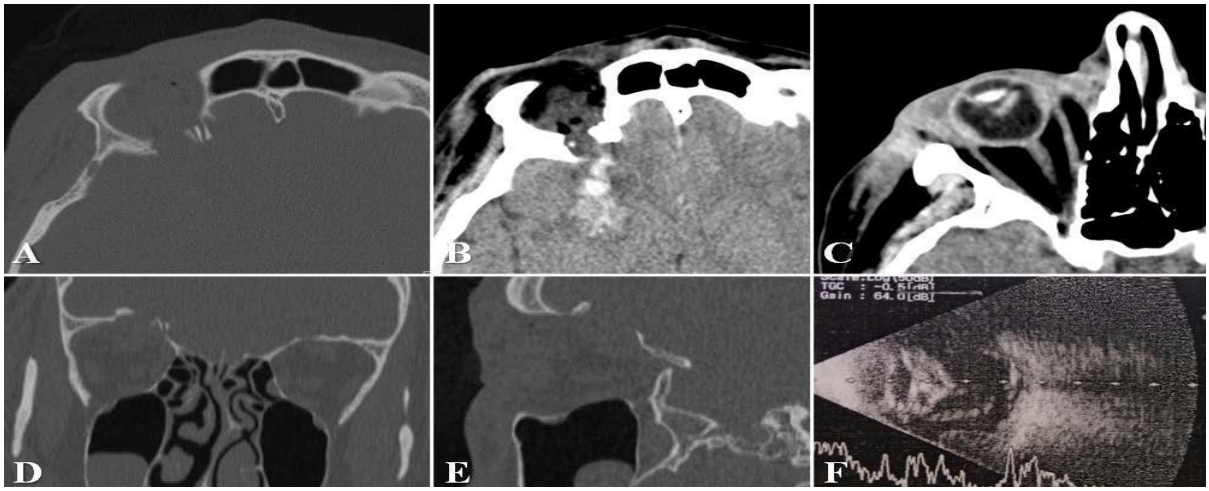

Figure I.87 - Fracture in the right orbital roof (CT-Scan) (A, B, D, E). Vitreous hemorrhage and choroidal hematoma (right eyeball) (C). Retinal detachment with vitreous hemorrhage and choroidal hematoma (ocular ultrasound) (F) (Turliuc *et al.*, 2017a).

In this case, the foreign body was upward directed and perforated the roof of the orbit, entering the cranial cavity and injuring the frontal lobe (Figure I.87, A-E) (Turliuc *et al.*, 2017a). Of all the orbital traumas with high speed of movement, the most common are when the patient falls into the foreign body, which has an upward direction (Mzimbiri *et al*., 2016).

The patient tends to perform a defensive movement, moving the neck back to protect itself from the blunt foreign body (Mzimbiri *et al*., 2016; Scarfo *et al*., 1990).

In conclusion, orbital injuries with blunt bodies should raise the suspicion of an intracranial trauma, even if the patient has minimal ocular and neurological symptoms, which may delay the identification of intracranial complications, leading to higher morbidity and mortality (Figure I.88 A and B) (Turliuc *et al*.,2017a).

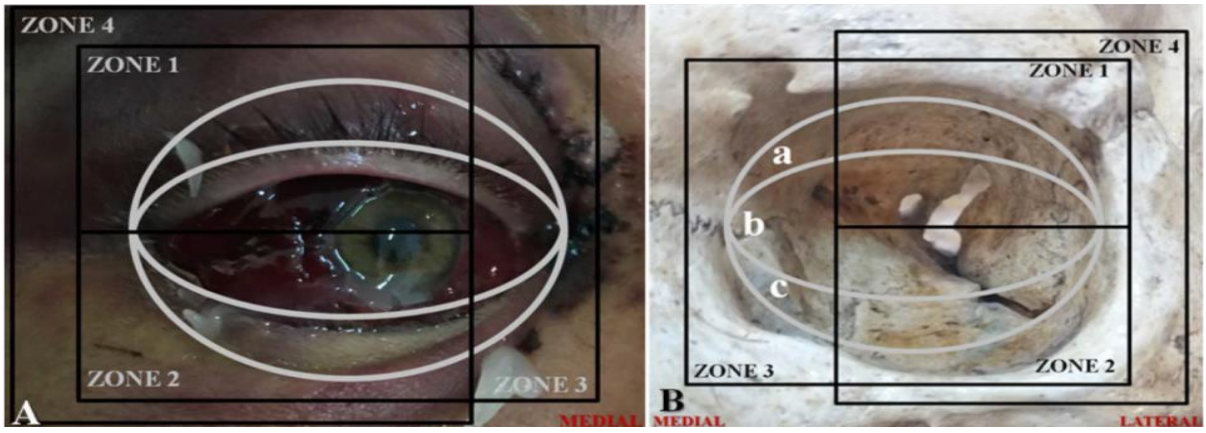

Figure I.88 - Turbin's diagram of ocular surface applied to our patient (A). Schematic diagram of ocular surface, adapted after Turbin *et al*., 2006 (B) (Turliuc *et al.*, 2017a).

Clinical Case No. 2.

The second case of penetrating orbital trauma was that of a 12-year-old boy who suffered a craniofacial trauma due to a fall accident while playing with another child. During the impact with the ground, it hit a piece of wood 6 cm long and 9.5 cm wide, which penetrated its orbit through the lower eyelid, towards the orbital apex (Figures I.89-I.91) (Turliuc *et al*.,2015a)

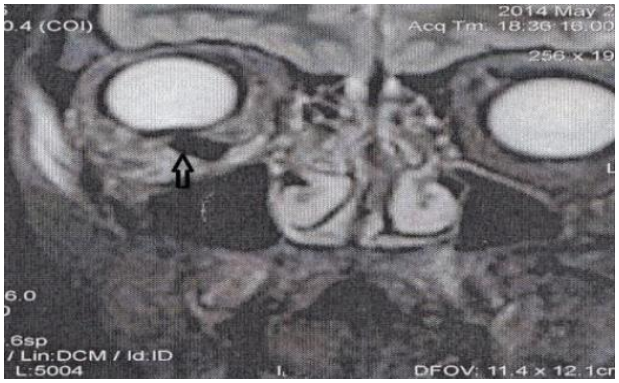

Figure I.89 - Coronal T1WI MRI showing the intraorbital foreign body (black arrow) (Turliuc *et al.*, 2015a).

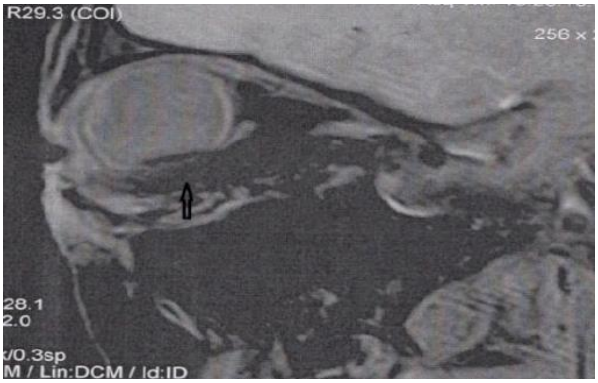

Figure I.90 - Sagittal T1WI MRI showing the intraorbital foreign body (black arrow) (Turliuc *et al*., 2015a).

Clinical ophthalmologic examination revealed the outer part of the wood fragment below the right lower orbital margin, eye pain and marked palpebral edema (Figures I.91 and I.92). Visual acuity was normal in both eyes. The craniocerebral MRI examination revealed an orbital foreign body, without damaging the optic nerve and blood vessels (Figures I.89, I.90 and I.93).

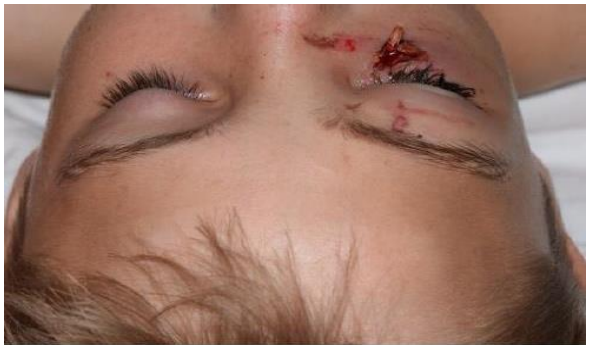

Figure I.91 - Photography of the patient at the first examination: a wooden foreign body, below the right lower orbital rim (Turliuc *et al.*, 2015a).

Figure I.92 - Postoperative results (Turliuc *et al*., 2015a).

The postoperative evolution of the patient was favorable (Figures I.92 and I.94) (Turliuc *et al*.,2015a).

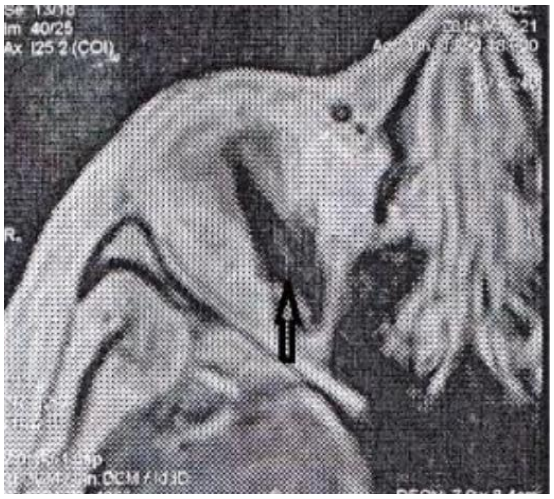

Figure I.93 - Axial T1WI MRI showing the intraorbital foreign body (black arrow) (Turliuc *et al*., 2015a).

Figure I.94 - Wooden foreign body extracted from the orbit (Turliuc *et al.*, 2015a).

Discussions

Intraorbital foreign bodies located in the anterior 2/3rd of the orbit can be approached extracranially, while those located at the orbital apex in the medial region of the optic nerve require a transcranial approach (Turliuc *et al*., 2015a).

Conclusion

This clinical case was presented because a large plant (wood) foreign body penetrated the orbit along its entire length to the apex, but surprisingly did not cause damage to vital structures, the eyeball, extraocular muscles and optic nerve.

Imaging diagnosis and emergency surgery influence the evolution and prognosis in terms of visual acuity (Turliuc *et al.*, 2015a).

I.5. Heuristic versus algorithmic thinking in neuro-ophthalmology

Background

Anatomy is considered the oldest child of Mother Medicine (Tubbs, 2014), and it has been the basis for all the medical fields. The efforts of anatomists during the time, to identify and describe anatomical structures were recognized as important contributions to medical fields (Kataoka *et al.*, 2007).

The optic chiasm structure and its discovery by the anatomists from ancient times till in the twentieth century was the research subject I published in an ISI ranked journal and the data are presented below. The data from my research on sphenoid bone were published in the journal ISI Web of *Science Anatomical Science International* in Springer Publications Group, which is summarized also below. The outcomes of the researches of the optic chiasm and sphenoid bone were published in the following articles:

Costea CF, Turliuc Ș, Buzdugă C, Cucu AI, Dumitrescu GF, Sava A, Turliuc MD. The history of optic chiasm from antiquity to the twentieth century. *Childs Nerv Syst*, 2017b, 33:1889–1898, IF=1.235.

Costea CF, Turliuc S, Cucu AI, Turliuc MD. To be or not to be Wilbrand's knee? A question that is looking for an answer. *Child's Nervous System*, 2018 a, 34(11):2135, IF=1.235.

Costea C, Turliuc S, Cucu A, Dumitrescu G, Carauleanu A, Buzduga C, Sava A, Costache I, Turliuc D. The "polymorphous" history of a polymorphous skull bone: the sphenoid, *Anatomical Science International*, 2018 b, 93(1): 14-22. IF=1.566

I.5.1. Theories about optic chiasm

During antiquity in ancient Greece, Hippocrates of Kos (ca. 460–370 BC), also considered the "father of medicine," studied and attempted to establish the function of the optic nerves and optic chiasm (Panourias *et al.*, 2012). He noted that a blow on the eyebrow area causes visual acuity to decrease and can even lead to blindness (Sarkies *et al.*, 2004). Thus, Hippocrates described the first case of craniofacial trauma that caused trauma to the optic nerves (Chandwick and Mann, 1950). He believes that the optic nerves and the optic chiasm can play an important role in transporting waste products from the brain to the eyeballs, and he also believes that tears are secreted by the brain (Hirscherg and Blodi, 1982; Murube, 2011). Hippocrates' theories could not be verified, because in ancient Greece, human body dissections were forbidden (Chandwick and Mann, 1950). Herophilus (ca. 330–260 BC) and Erasistratus (ca. 330–255 BC) attempted to prove the same theory of optic nerves and chiasm as Hippocrates. They were the first to perform dissections on human bodies and animals and to document various anatomical structures (Finger, 1994; Reevesard Taylor, 2004; Loukas *et al*., 2011). Rufus of Ephesus (80–150 AD) was the first anatomist to perform dissections in Alexandria and was interested in the neuroanatomy of the sellar region (Ellenbogen *et al.*, 2012). He described the role of optical chiasm in the visual system and also understood the ventricular system (Goodrich *et al.*, 2012; Howard, 2012).

New theories on optic chiasm at the beginning of the thirteenth century

Mondino de Luzzi (ca. 1270-1326) wrote the first anatomy textbook in 1316, "Anathomia corporis humani," in which he mentioned various dissection techniques (DeLuzzi, 1316; Crivellor and Ribatti, 2006). In his book, he mentioned that optic chiasm is a common station for optic nerves (Swanson,2014). In the 7th illustration, labelled by D, Mondino showed optic nerves, that came from the forward ventricles, joined together to form the chiasm and extended as to enter into eyes. In his vision, if one eye was closed, the whole spirit was transferred to the other eye (De Luzzi, 1316).

Leonardo da Vinci's view on the optic chiasm **-** In Italy, dissections on human bodies began after 1000 years for forensic purposes, but also for the study of medicine. Leonardo da Vinci (1452–1519) was considered the greatest anatomical illustrator of the time (McMurrich, 1930). He performed dissections in hospitals in Milan and Rome (Nanda, 2016) totalling approximately 30 dissections during his lifetime (Calkins *et al*., 1999). He was first one to draw the diagram of the cranial nerves, but also that of the optical chiasm (Figure I.95) (Goodrich, 2000; Goodrich, 2012; Mortazavi *et al.*, 2014). He considered that optic chiasm was involved in the process of vision, but also in the mechanism of conjugated movement of the eyeballs (Wade, 1999; Howard, 2012). Leonardo da Vinci made no mention of the crossing of optic snow at the level of the chiasm (Rucker, 1958).

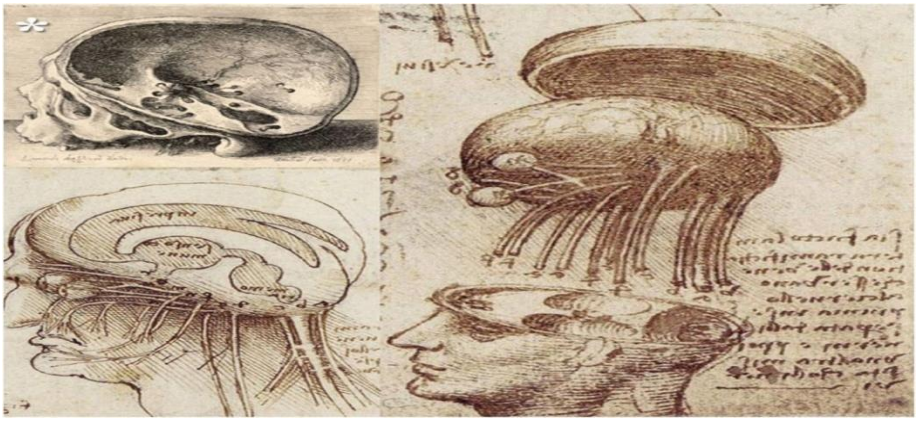

Figure I.95 - The optic chiasm in Da Vinci's vision (asterisk indicates the reprint of Leonardo da Vinci of Wenceslaus Hollar, 1607– 1677) (public domain).

New theories on the structure of the optic chiasm in the fifteenth, sixteenth and seventeenth centuries

The anatomists Bartolomeo Eustachio, Berengario da Carpi, Constanzo Varolio and Andreas Vesalius were the most famous in the sixteenth and seventeenth centuries. In 1543, Vesalius published his book "De Humani Corporis Fabrica", in which he described the

structure, origins and trajectory of the optic nerves to the level of the optic chiasm (Costea *et al.*,2017 b). The first to correctly identify the optic pathways and correctly describe the optic chiasm was the neuroscientist of the Renaissance, Bartolomeo Eustachio (1524–1574). He contradicted the theory of the Greeks, who believed that optic nerves projected into the lateral ventricles.

Eustachius considered that the optic nerves project into the lateral geniculate nuclei passing posterior to the thalamus (Howard and Rogers, 1995). He published in 1552 "Tabulae anatomicae" in which he drew the optic chiasm and the base of the skull (Finger, 1994; Barone, 2007).

René Descartes (1596–1650), a renowned French philosopher, agreed with his predecessor's theory of binocular vision, but considered that optic nerves do not intersect in the optic chiasm. In his book "Dioptrique" (1637) in one of the diagrams he drew the ipsilateral projection of the optic nerves that met in the brain and then combined in the pineal body. At the level of the optic chiasm, in his vision the optic nerves did not intersect (Figure I.96, a) (Costea *et al*.,2017b).

Shaping the idea of partial decussation of the optic chiasm in the eighteenth and nineteenth centuries

Although the trajectory of the optic nerves was controversial in the seventeenth century, Isaac Newton (1642–1727) was the first to claim that the optic nerves partially intersect at the level of the chiasm.

His attention on the optic chiasm appeared on 15 March 1682, when his friend, the physician, William Briggs (1650–1704) presented a paper about the optic nerves, optic chiasm and vision.

He would also explain Isaac Newton later the notions of their anatomy and how the eyes are dissected. Briggs presented his lecture "A New Theory of Vision", in front of the Royal Society of London (Briggs, 1809), in which he mentioned the theories of his forerunners, stating that the optic nerves do not cross in the optic chiasm (Briggs, 1809).

In the second half of the seventeenth century, neuroscientists studied the path of optic nerves beyond the optic chiasm (Costea *et al*., 2017b).

Isaac Newton, in 1704 in his book “O ptics”, correctly presented the theory that the fibers of the optic nerves coming from the nasal half of the retina pass to the opposite side, forming the optic chiasm, while the fibers from the temporal half extend to the brain on the same side (Figure I.96,b), (Y Cajal, 2002).

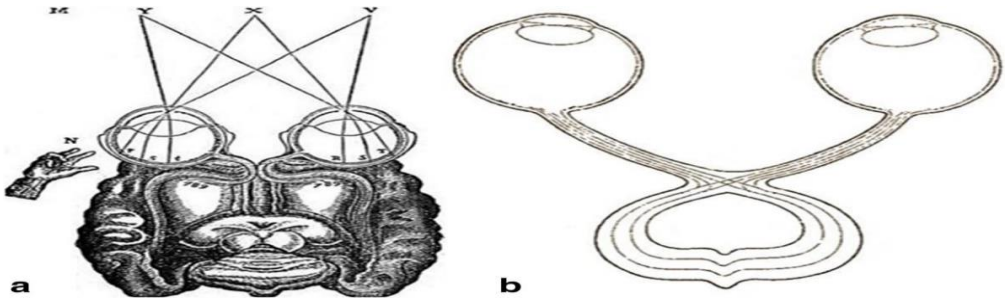

Figure I.96 – a. The optic chiasm and the eye in the vision of René Descartes in "Dioptrique",1637. b. Adaptation after schematic illustration of the optic chiasm by Isaac Newton, 1704 (public domain).

In Europe the physicians introduced in the dayly medical practice the theoretical notions about the function of the optic chiasm (Costea *et al*.,2017b). In the eighteenth century, Newton was followed by other researchers with similar views. The founder of modern neuroscience, Santiago Ramón Y Cajal (1852–1934) (Figure I.97,a) discovered that in mammalian chiasm, only a few axons of the optic nerves intersected (Cajal, 1899; Cajal, 1911).He used Marchi and Golgi staining method, in dissections on rabbits and cats, as well as the staining with methylene blue to show the existence of uncrossed and crossed fibres in the optic chiasm, presenting the highly representative schematic figures (Figure I.97,b) (Y Cajal, 1911; Guillery, 1982). In Cajal's view, the brain could not operate with a disrupted sensory space, seeing chiasm as a device for correcting inversion of the visual field produced by the crystalline lens in the eyes (Guillery,1982). After 1950, Polyak, Hoyt, and Luis, after experiments on macaque monkeys, provided informations that complemented theories of optic chiasm (Polyak, 1957; Hoyt and Luis O., 1962).

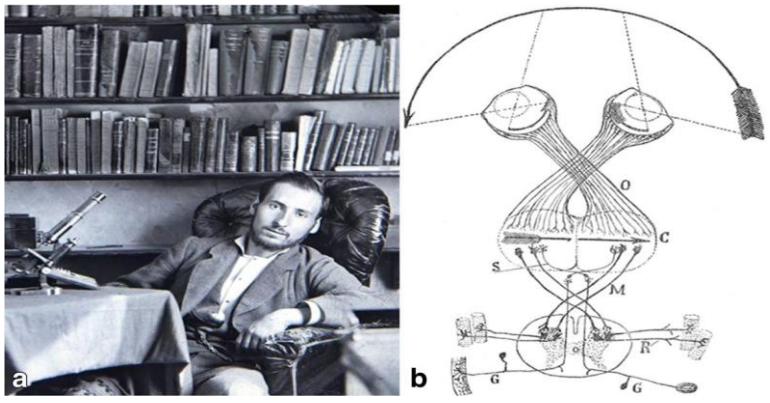

Figure I.97 – a. Santiago Ramón Y Cajal (1852–1934), one of the founders of modern neuroscience. b. Schematic illustration of the chiasm drew by Santiago Ramón Y Cajal (public domain).

Conclusions

Optic chiasm has fascinated anatomists over the centuries, who have developed various theories about its anatomical structure and function. In the nineteenth century, following numerous vivisections, anatomists reached the pinnacle of understanding the

anatomical structure and functions of the optic nerves, the optic chiasm, and their relationship to the brain. All these theories opened a new path for the formation of a new discipline such as neuro-ophthalmology, which developed in the twentieth century (Costea *et al*.,2017b).

I.5.2. Debates in neuro-ophthalmology on Wilbrand's knee

In 2018, my collegues and I published an article (letter to the editor) on the presence of Wilbrand's knee at the request of Prof. Dr. Concezio De Rocco, President of the International Society for Pediatric Neurosurgery and the European Society for Pediatric Neurosurgery and Editor-in-Chief of the Child's Nervous System journal. The data discussed in this article are set out below. German ophthalmologist Herman Wilbrand (1851-1935) (Figure I.98) observed that in the optic chiasm, nerve fibers from the lower quadrant of the retina loop forward into the termination of the contralateral optic nerve before going back into the optic tract (Figure I.99) (Wilbrand,1926). In 1997, Horton showed that the fibers of the optic nerve intersect at the optic chiasm, but without entering the contralateral optic nerve (Horton, 1997). His study was continued by Lee *et al*. to the same conclusion (Lee *et al*., 2006b). These two studies debated the presence of Wilbrand's knee (Costea *et al*., 2018a). In 2014, Shin *et al.*, also continued research using anisotropic light-reflecting properties of myelinated axons and analyzed Wilbrand's knee structure on 25 μm sections (Shin *et al*., 2014).

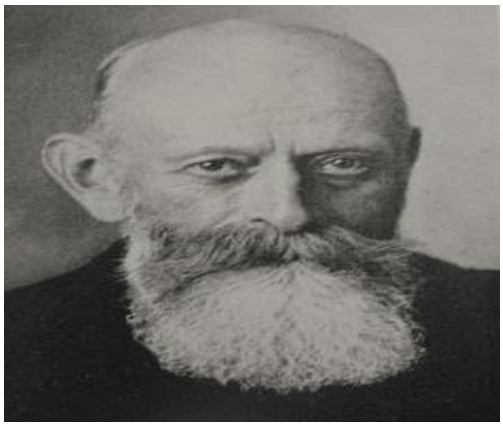

Figure I.98. Hermann Wilbrand (1851-1935) (public domain).

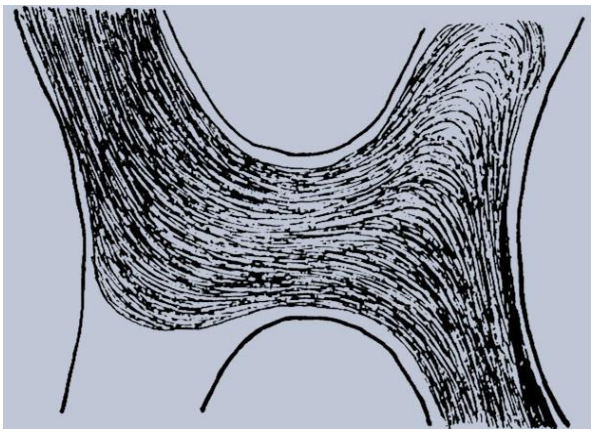

Figure I.99. Final anatomic drawing by Wilbrand of his "knee" (adapted after Wilbrand, 1926).

Researchers at Georgetown University and in the University of Maryland studied optical chiasm in three monkeys and four humans and found that human optical chiasm specimens contained fiber tracts corresponding to Wilbrand's knee. Wilbrand's knee fibers were not identified in monkeys optical chiasm specimens (Kachela *et al*.,2016). Considering the dispute in the literature regarding the presence of Wilbrand's knee, we believe that, despite conflicting reports, the Professor of Ophthalmology Hermann Wilbrand is worth

mentioning in all papers on the history of neuro-ophthalmology, for his important concept – Wilbrand's knee, and for his important contribution: the beginning of modern neuro-ophthalmology (Costea *et al*., 2018a).

I.5.3. Researches regarding the skull base

Background

Anatomists have discovered and researched over time various bones, muscles, and vascular structures, making a major contribution to scientific research in the field of anatomy (Kataoka *et al*. 2007). Located at the base of the skull, the anatomical structure of the sphenoid bone was a challenge for anatomists, especially due to its anatomical position. The history of the anatomical description and the names of the component parts of the sphenoid bone is complicated but fascinating (Costea *et al*., 2018,b).

I.5.3.1. Anatomical conformation of the sphenoid bone

The sphenoid bone is an unpaired, symmetrical bone arranged medially in the middle of the base of the skull. It has had various names over time: "cuneiform bone" by the Romans (Sawai, 2008), the "sphenoid" by the Greeks, "os colatorii" by the Arabs, and the "basilar bone" by the barbarians (du Laurens 1621).

The structure of the bone has been described since antiquity, even if only its external part was mentioned (Lopez - Serna *et al*., 2012). Galen of Pergamon (129–200), considered the father of anatomy, was the first to describe the anatomy of the sphenoid, comparing it to a wedge, and this similarity was the origin of the term "sphenoid', from *σφήνα* Greek (*sfina*), meaning "wedge," and *οιδοσ* Greek (*oidos*), meaning "similar to" (Galenus, 1630).

Later Andreas Vesalius (1514-1564), was considered by Riva *et al*. (2010) to be the ''author of the anatomical revolution'' describing almost entirely the structure of the sphenoid bone (Riva *et al*.,2010). He used the term ''cuneiform bone'' for the sphenoid bone, and described it as a "polymorphous" bone that was unpaired and formed part of the skull cavity containing the brain (Riva *et al*.,2010). Vesalius observed that the cuneiform bone (the sphenoid) looked like a "flying bird" (Figure I.100, A-C) (Vesalius 1555).

Another famous professor of anatomy and surgery Gabriele Fallopius, made numerous dissections on fetuses, children and adults, corrected and commented on numerous statements about the anatomical structures mentioned by Vesalius in his book "De Humani Corporis Fabrica", including them in his book, "Observationes anatomice" (1561), making important contributions to the description of the anatomy and embryology of its sphenoid bone (Fazekas and Ko´sa 1978).

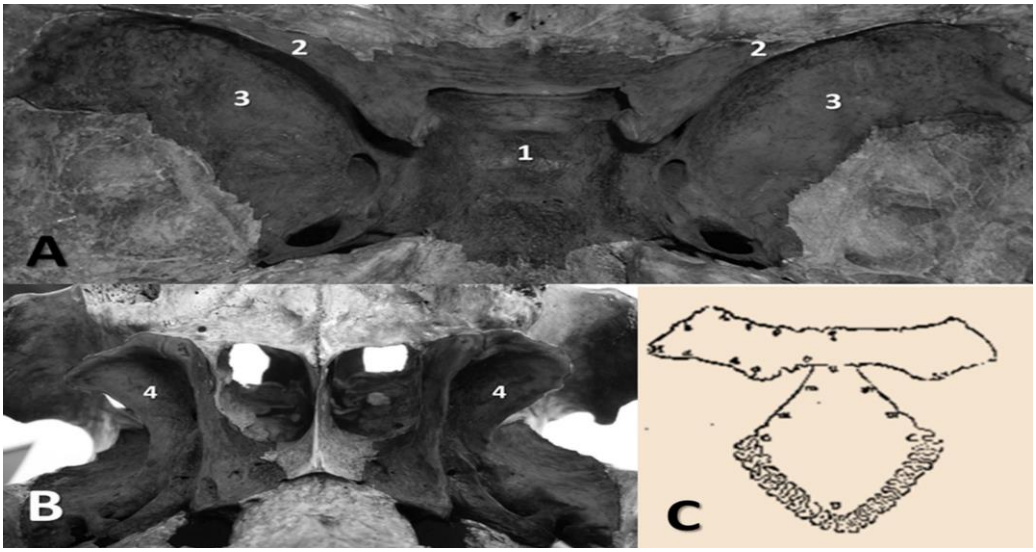

Figure I.100 – A. Endocranial view of the sphenoid bone, showing the body (1), lesser wings (2), and greater wings (3). B. Exocranial view of the sphenoid bone with the pterygoid processes (4). C.Vesalius's drawing of the sphenoid bone contour (adapted from "De humani corporis fabrica", 1555) (Costea *et al.*,2018b).

The wings of the sphenoid bone - Initially, ancient anatomists differentiated only the body from the large wings of the sphenoid (Cloquet and Knox 1828), comparing them to the wings of a bat or a bird (Costea *et al.* 2018,b).

Vesalius described the great wings of the sphenoid bone in detail, but the first distinct description of it was made by Giovanni Filippo Ingrassias (1510–1580), Vesalius's student and later a professor at the University of Naples and Protomedicus of Sicily, gave the first distinct account of the true configuration of the sphenoid bone (Craigie 1838).

I.5.3.2. The sellar region of the sphenoid bone

When Galen studied the sphenoid bone, he identified and described the pituitary gland on the upper face of the sphenoid bone body.

He found that this structure was located outside the dura mater and described the depression where the pituitary gland was located at the sphenoid, formulating the theory that brain activity is discharged through this depression in the sphenoid bone (ie, the Turkish sella) and the cribriform plate as phlegm (Greenblatt *et al*. 1997, Johnson and Green, 2014).

In 1543, Vesalius described the anatomy of the entire sellar region (Figure I.101, A-D) of the inner part of the cuneiform bone which had a wide depression containing a gland (the pituitary gland), in which phlegm flowed from the brain (Vesalius, 1555) and called this depression the sinus.

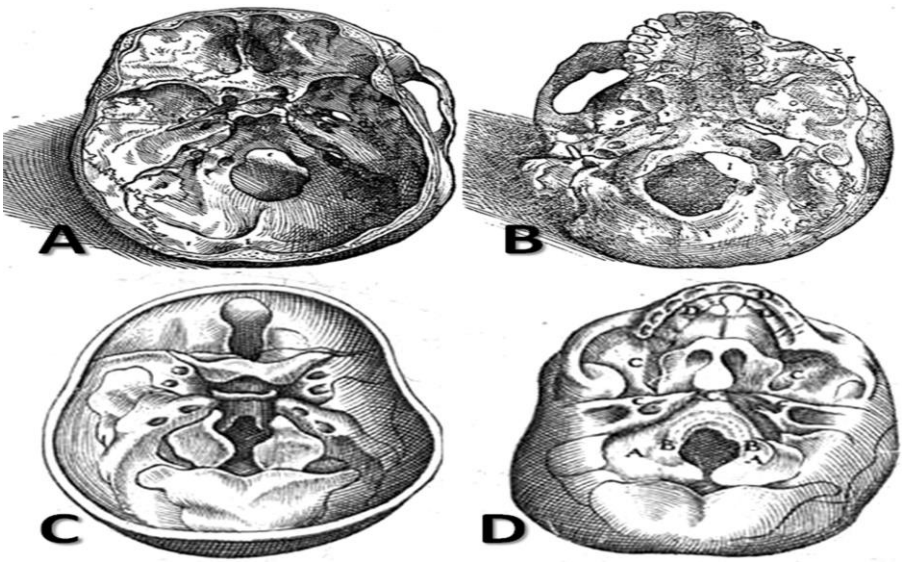

Figure I.101, A–D. Drawings of the skull base highlighting the sphenoid bone in Vesalius's "De humani corporis fabrica" (1955) (A and B) and in du Laurens' "Historia anatomica humani corporis" (1600) (C and D).

He criticized Galen, saying that his opinion on these structures was totally wrong and that the depression of the sphenoid bone was not perforated "like a sponge or a sieve" as Galen thought (Vesalius, 1555). In 1559, the Italian anatomist Realdo Columbo (1515–1559) ignored the term cuniform bone and used the term sphenoid bone. He also noticed a similarity (sellæ simillimum) between the depression in the sphenoid and "a chair", which led him to introduce the term sella for this depression. Just like Vesalius, Columbo corrected Galen's error regarding the existence of numerous foramina in the sphenoid bone (Columbo 1559). Later, the Flemish physicist and botanist Adrianus Spigelius (1578–1625), one of the most eminent anatomists at the University of Padua, described the saddle-shaped depression in its thickest region, comparing it with a Turkish saddle: "extuberantibus, qui cum ossis crassam partem cingant, ephippio non absimilem, Sella turcica a forma dicuntur" ("the protuberances, which are said to be in the shape of a Turkish saddle, because they surround the thick part of the bone, not unlike a saddle") (Spigelius 1627).

The term Turkish saddle (Latin) was introduced by Spigelius in his book "De humani corporis fabrica libri decem" (published in 1627, two years after his death) (Costea *et al*., 2018,b). A group of Turkish medical historians (Tekiner *et al.* 2015) recently presented analogies between the Turkish saddle of the seventeenth century and the Turkish saddle of Spigelius. The sella turcica has three parts.

The first is the tuberculum sellae, the slight anterior elevation on the body of the sphenoid bone, which corresponds to the pommel (the upward-curving or upward-projecting part of a saddle in front of the rider). The second part is the hypophyseal fossa, which hosts

the hypophysis and resembles the seat of the saddle. The third part is the dorsum sellae, which is similar to the cantle - the raised, curved part at the back of the saddle (Figure I.102 A and B) (Tekiner *et al*. 2015). In 1998, several terms were used for saddle-shaped depression on the sphenoid (including "sella turcica',' "sella equina',' "ephippium", "sella sphenoidis" and "Turkish saddle"), the Federative Committee on Anatomical Terminology FCAT selected "sella turcica" as the official Latin and English term for this anatomical structure to promote international consistency in nomenclature (Federative Committee on Anatomical Terminology – FCAT,1998).

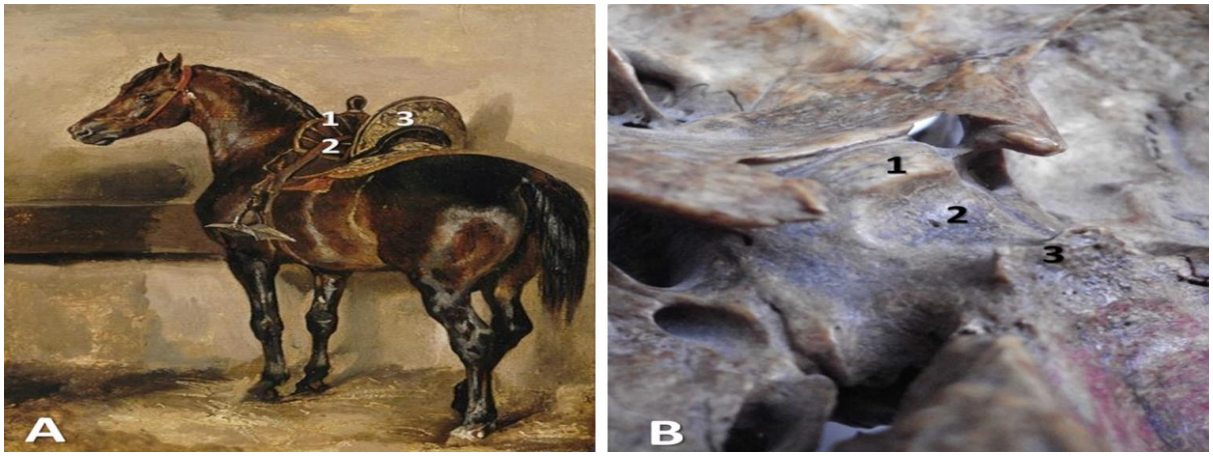

Figure I.102 A. - Turkish Horse in a Stable by Theodore Gericault (1791–1824); note the labelled parts of the Turkish saddle: pommel (1), seat (2), and cantle (3) (public domain). B. - The anatomy of the sella turcica: tuberculum sellae (1), hypophysial fossa (2), and dorsum sella (3) (Costea *et al*.,2018b).

Clinoid and pterygoid processes

The pituitary fossa or sella turcica is surrounded by four bony prominences (called the clinoid processes or apophyses), two anterior ones (forming parts of the lesser wings), and two posterior ones (where the cerebellar tentorium inserts). Andreas Vesalius observed these prominences and called them "clinoid processes" (Vesalius 1555).

It resembled the wings of a bat, being thin, two on each side attached to the sphenoid bone (Vesalius, 1555).

The anatomical knowledge of the morphology and structure of the sphenoid bone acquired by anatomists during the Renaissance influenced Baroque painters, who wished to accurately represent the human form (Kemp 2010).

Indeed, soon after Vesalius's remarkable works on the human cranium and consequently the sphenoid bone, socio-political events of the period led the artistic community to adopt the skull as a symbol of the impermanence of life (Figure I.103, A and B) (Costea *et al*.,2018b).

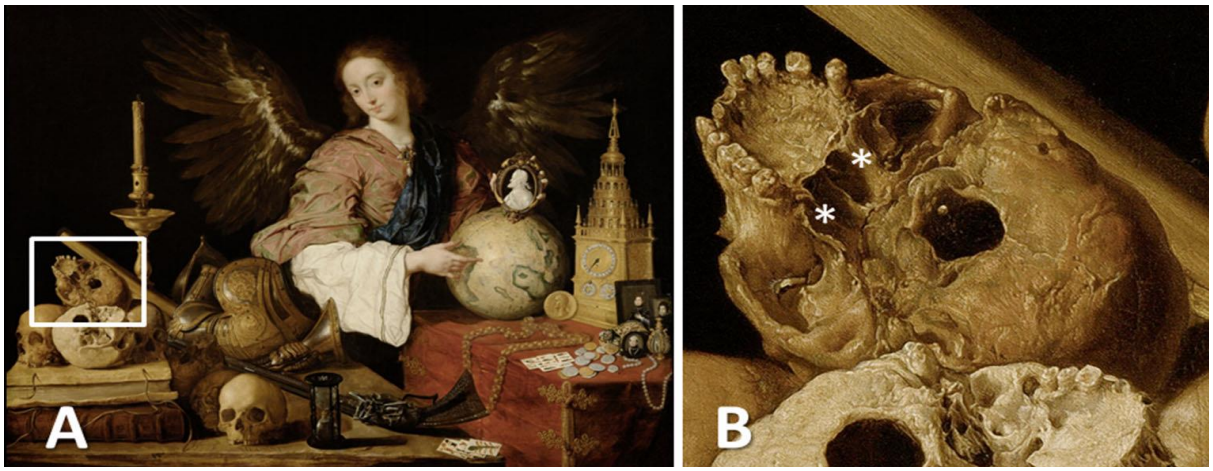

Figure I.103 A. - Vanitas by Antonio de Pereda y Salgado (1632–1636). B.- Detail showing the pterygoid processes (indicated by asterisks) of the skull depicted in the painting (public domain).

Foramina and bone impressions

Vesalius in his book “De Humani Corporis Fabrica” (Book VII) described the foramina at the level of the sphenoid bone in detail with the aid of appropriate drawings (Vesalius 1555). He used the letters of the alphabet to highlight them in his drawings, e.g. "H" for foramen rotundum, "Q" for foramen ovale, and "R" for foramen spinosum (Vesalius 1555). The letter "S" was used by Vesalius to denote a distinct foramen but inconsistently located between the foramen rotundum and the foramen vesalii (Hoblyn,1865).

The sphenoid sinus

Berengario da Carpi (1460–1530) in his work "Isagogae breves in anatomiam humani corporis" described the sutures of the sphenoid bone with the other neighboring bones (Ball, 1910). He was the first to mention sphenoid sinuses (Skinner, 1961) which later became the most varied cavities in the human body (Teatini *et al*., 1987).

Vesalius, however, gave the clearest information about the sphenoid bone, noting that a lower part of the body of the sphenoid had two cavities that he called antra and were separated by a bony septum, similar to a wall in the middle of a house (Vesalius 1555). Spigelius vaguely referred to these sphenoid cavities (Spigelus 1627).

Conclusions

Due to the polymorphous structure of the sphenoid bone, there have been many anatomists - over the course of millennia - who have made valuable contributions to our knowledge of this structure in the human body. Certainly if there was no passion for these anatomists to discover new anatomical structures, there would be no anatomy of the base of the human skull (Costea *et al*.,2018b).

I.6. The eye of the mind – neuro-ophthalmological pathology

I.6.1. Researches regarding meningiomas related to the Chernobyl irradiation disaster in north-eastern Romania

Background

More than 30 years have passed since the Chernobyl nuclear accident, and more than 30,000 articles have been published, most in Slavic language (Yablokov *et al*.,2009), and the exact real impact on the health of the world's population is still unknown (Cucu *et al.*, 2018c). The explosion of reactor four at the Chernobyl nuclear power plant took place on April 26, 1986, and the radioactive cloud spread to Europe (France, Italy, Germany, Greece, Austria, Switzerland, Slovenia, Scandinavia, Iceland) and Asia (Turkey, Georgia, Armenia, China), North Africa and North America (Yablokov *et al*.,2009). Romania was also affected by the radioactive cloud, with high concentrations of radiation in the Eastern and Southern part of the country (Constantinescu and Bugoi,1999).

Nevertheless, the most significant radioactive substances in the emissions were Iodine, Strontium, Caesium and Plutonium, each with different half-lives and with its own set of problems (Constantinescu and Bugoi,1999). Meningioma is the most common radio-induced tumor that occurs in the adult population, literature describing over 150 individuals with radio-induced meningiomas (Pettorini *et al*.,2008). Several hypotheses have suggested the role of high doses of radiation in the development of meningiomas, a study in 1999 reporting an increased incidence of meningiomas in survivors of the Hiroshima atomic bombing, even finding a significant correlation between tumor incidence and the dose of radiation, and with a higher incidence of meningiomas in hypocenter areas (Shintani *et al*.,1999).

I published together with my neurosurgeonts collegues an article about the impact on the incidence of meningiomas after the Chernobyl irradiation disaster in north-eastern Romania, and the researches data are presented below:

Cucu AI, **Costea CF**, Carauleanu A, Dumitrescu GF, Sava A, Scripcariu IS, Costan VV, Turliuc S, Poeata I, Turliuc DM. Meningiomas Related to the Chernobyl Irradiation Disaster in North-Eastern Romania Between 1990 and 2015, *Revista de Chimie*, 2018c, 69(6): 1562-1565. IF=1.605.

In this article we presented the tumor behavior of intracranial meningiomas in the North-East of Romania affected by the radioactive cloud from Chernobyl, over a period of 25 years, between 1990 and 2015. We conducted a retrospective cohort-based study on a group

of 1287 patients admitted to the "Prof.Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania, diagnosed with intracranial meningiomas and operated between 1990 and 2015.We included in the study, grade I, II and III intracranial meningiomas and the exclusion criteria were age under 18 and those associated with genetic syndromes (e.g. neurofibromatosis), (Cucu *et al*.,2018c).

Results and discussions

Of the 1287 patients, 64.65% (832 cases) were women and 35.35% (455 cases) were men. Meningiomas were predominant in the 50-59 age group (30.30% of patients), followed by the 60-69 age group (28.36% of patients) (Cucu *et al.*,2018c). Of all 1287 meningiomas, 79.80% (1027 cases) were grade I and 20.20% (260 cases) were grade II and III. In the 1278 cases 51.20% of cases were diagnosed in the first 12 years of 1995-2006 and the remaining 48.74% were diagnosed and operated in the next 9 years (Figure I.104) (Cucu *et al*., 2018c). Regarding the influence of radiation from the Chernobyl accident on the development of brain tumors, further studies have shown a significant correlation. Thus, Orlov *et al*. (2001,2006) proved that between 1987 and 2004, in Ukraine, the incidence of CNS tumors in children up to 3 years of age doubled, and in infants increased by 7.5 times (Orlov *et al*.,2001; Orlov *et al*.,2006).

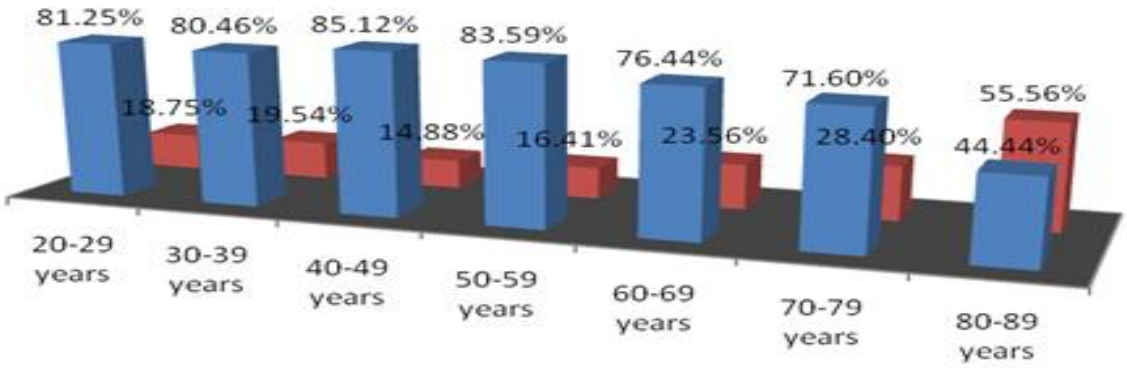

Figure I.104 - Distribution of cases by the degree of malignancy, according to age groups (1990-2015) (Cucu *et al*.,2018c).

Another study conducted on a cohort of Latvian and Estonian patients (5546 and 4786 men, respectively), followed from 1986-1998, showed an increase in the incidence of brain cancers (Rahu *et al*.,2006) and a report on the North-Eastern population of Romania showed the increase in the incidence of oligoastrocytoma cases after the accident (Dumitrescu *et al.*,1995). In our study we observed an increase in intracranial meningiomas between 1993-1996 and 2007-2015, i.e. 7-10 years and 21-30 years after the accident (Cucu *et al*.,2018c).
Also, a study conducted by the Ukrainian Institute of Neurosurgery in Kiev showed that the number of children with brain tumors increased by 63.7% between 1987 and 1991 compared

to the period before Chernobyl, i.e. 1981-1985 (Orlov *et al*.,1993; Orlov *et al.*,1995; Orlov and Sharevscy,2003). What we found in our study is that between 1995-2015, grade I meningioma indicates a variation in the number of patients, without showing a cyclicity, in contrast to grade II and III meningiomas, which were absent until 1995, with an upward trend between 1996-2000 (10-14 years after the accident), followed by a variation of these cases after 2000, with irregular increases and decreases in the diagnosis of these patients (Figures I.105 and I.106) (Cucu *et al*.,2018c).

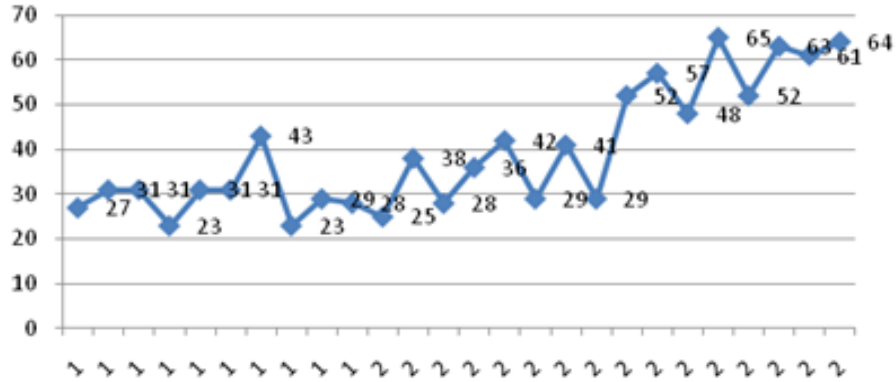

Figure I.105 -Evolution of the annual frequency of grade I meningiomas (Cucu *et al*.,2018c).

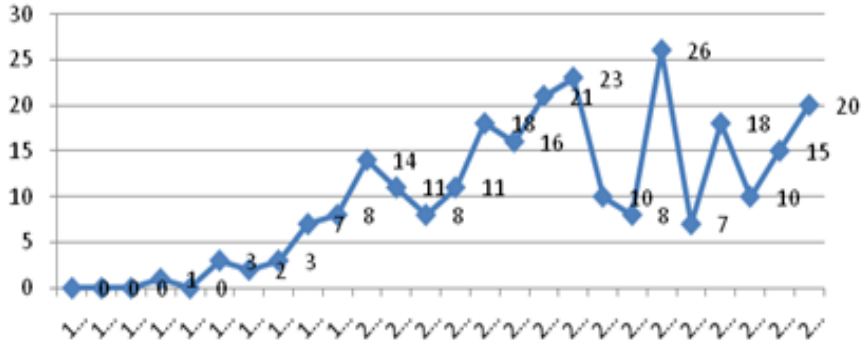

Figure I.106 -Evolution of the annual frequency of grade II and III meningiomas (Cucu *et al*.,2018c).

Conclusions

Between 1990 and 2015 there was an increase in intracranial meningiomas, with the first peak between 1993-1996 and the second peak in 2007-2015, corresponding to 7-10 years and 21-30 years after the Chernobyl accident.

The most numerous meningiomas were grade I, followed by grade II and grade III, except in the 80-89 age group.

Grade II and III meningiomas have an upward trend in the period 1996-2000, which corresponds to 10-14 years after the Chernobyl accident (Cucu *et al*.,2018c).

I.6.2. Researches regarding visual impairment in orbitofrontal and sphenoidal fibrous dysplasia

Background

Fibrous dysplasia (FD) is a rare disease characterized by enlarged bone mass (Ahe *et al.*, 2002) that develops in 71.9-86% of cases of postzygotic activating mutations of the GNAS gene on chromosome 20q13. It can affect a single bone structure or more (Schwindinger *et al.*, 1992; Tabarean-Delalon *et al.*, 2013; Weistein *et al.*, 2002). The prevalence of this disease varies between 10-25% in monostotic form and up to 90% in polyostotic form (Alam and Chander, 2003). FD most often manifests itself in childhood and adolescence, around the age of five (Burke *et al.*, 2017). Decreased visual acuity is progressive in FD due to bone excrescence and compression of the optic nerves (ONS) and can occur in 50-90% of cases (Katz and Nerod, 1998). FD may be associated with sphenoid sinus mucocele (Sfarifi *et al.*, 2013).

I published together with my neurosurgeonts collegues a research regarding a clinical case of orbitofrontal fibrous dysplasia with visual impairment in the following article:

Costea CF, Cucu A, Costan VV, Dumitrescu GF, Sava A, Turliuc D. Visual Impairment in Orbitofrontal and Sphenoidal Fibrous Dysplasia Associated with Sphenoid Sinus Mucocele, *Journal of Clinical Research and Ophthalmology,* 2015, 2(4): 54-57.

Clinical Case

I published the case of a 20-year-old male patient admitted to the Department of Neurosurgery of the "Prof. Dr. Nicola Oblu" Emergency Clinical Hospital Iași, Romania for facial deformity, headache and sudden progressive decrease in visual acuity in the right eye over the last years. Ophthalmologic examination showed a visual acuity of less than 1/500 in the right eye and 20/60 in the left eye. Examination of the eye fundus revealed atrophy of the right optic nerve and a normal appearance in the left eye. Imaging diagnosis was monostotic cranio-facial FD associated with sphenoid sinus mucocele causing partial obliteration of the bilateral optic canals (Figure I.107, A-D) (Costea *et al.*,2015c).

The patient underwent two successive operations for bilateral decompression of the right optic nerve and evacuation of the sphenoid mucocele by transfacial and transmaxillary approach (Figure I.108) (Costea *et al*., 2015c).

Histopathological examination confirmed the diagnosis of FD (Figures I.109 and I.110) (Costea *et al*., 2015c).

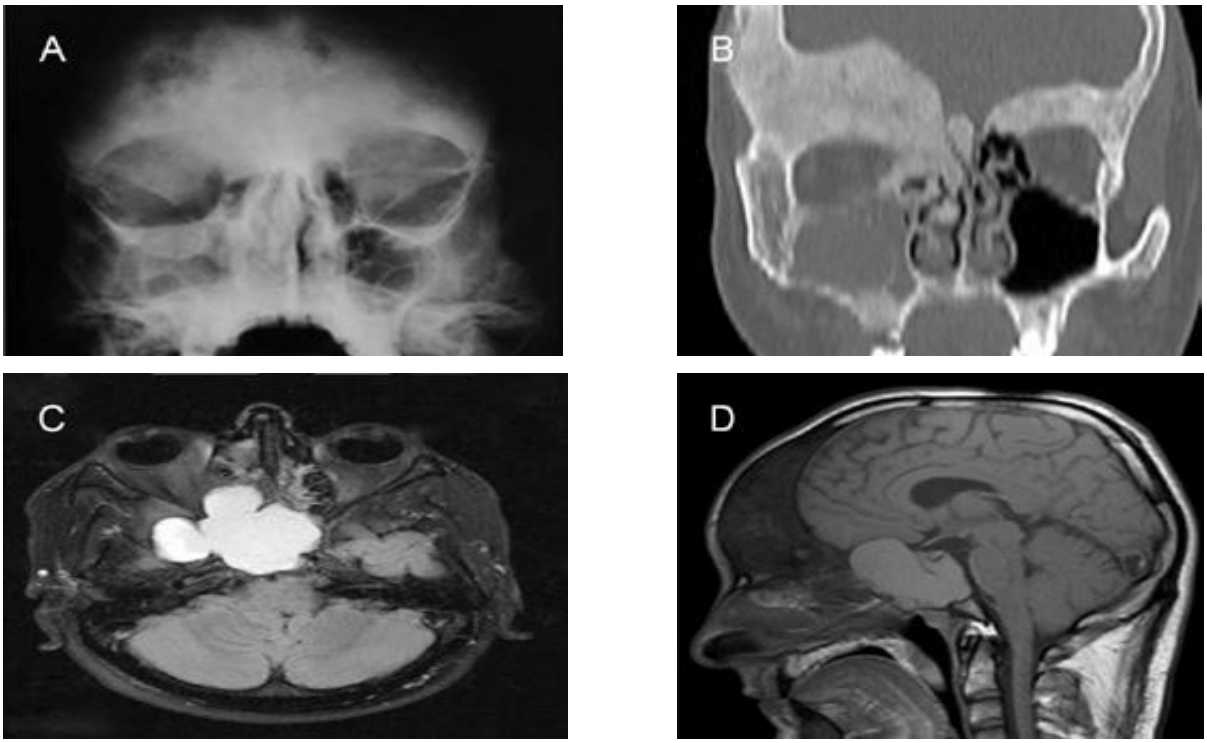

Figure I.107 - Orbitofrontal and sphenoidal fibrous dysplasia: A). Radiographic appearance characteristic aspects of "ground glass"; B). CT scan images;C) MRI scan images: homogeneous well defined cystic lesion (57/56/41mm) located in the sellar, suprasellar and parasellar areas, predominantly on the right side, extending in the anterior cranial fossa, with compressive effect on the optic chiasm; D). T1-weighted MRI scan showing the large cyst and frontal dysplasia (Costea *et al.*, 2015c).

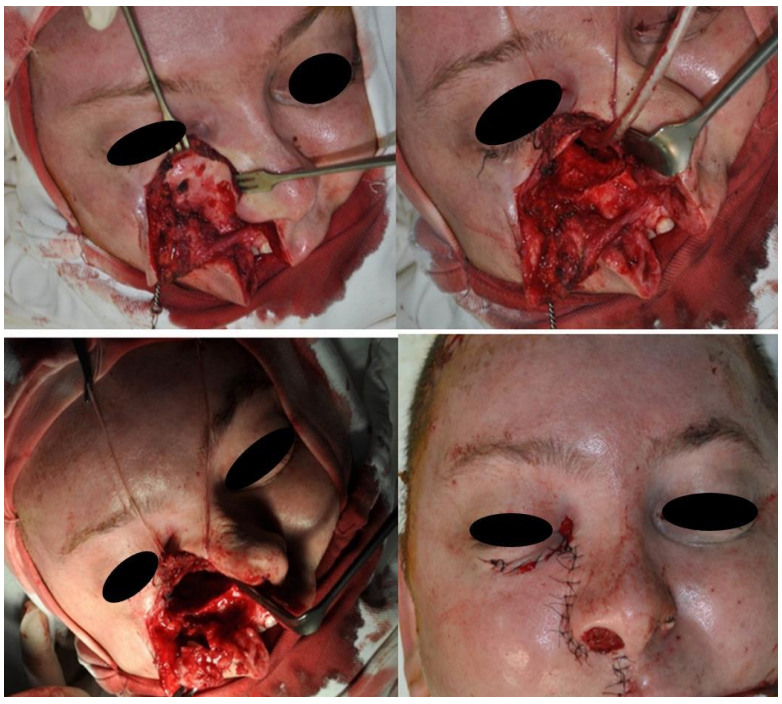

Figure I.108. Intraoperative and postoperative views - transmaxillary approach (Costea *et al*., 2015c).

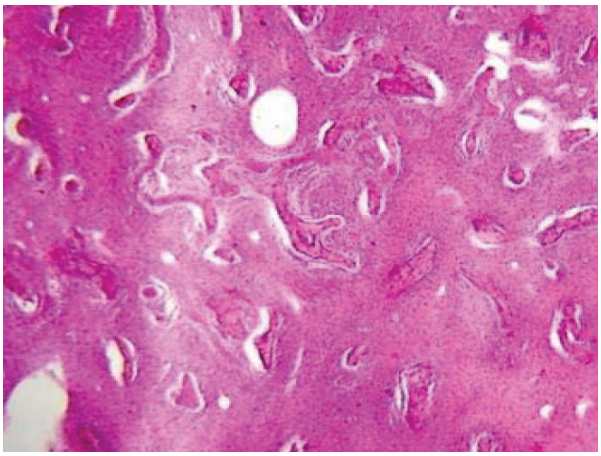

Figure I.109. Fibrous dysplasia: normal bone was replaced with newly formed bone trabeculae, thin, irregular-looking like "Chinese letters", separated by fibrous connective tissue (HE, x40) (Costea *et al.*, 2015c)

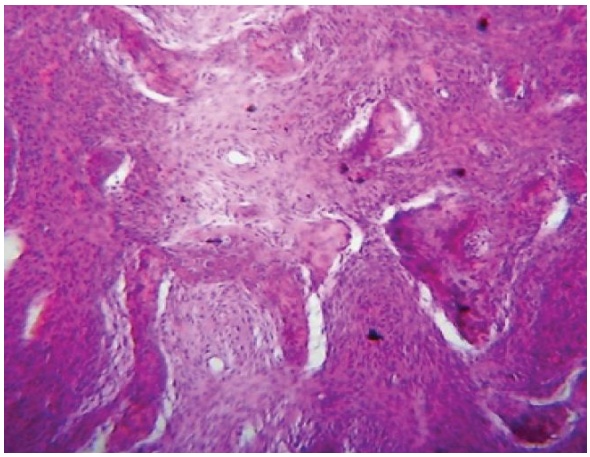

Figure I.110. Microscopic field with lens of higher power highlights immature bone blades, poorly calcified bone pattern and with the presence of osteoblasts only from place to place. Form of the newly formed bone blades ranging from small oval islands, curves or serpinginiouse, which gives the impression of "Chinese letters" or "alphabet soup" (HE, x100) (Costea *et al*., 2015c).

Disscusions

Regarding FD, the frontal and sphenoid bone are the least involved (Araghi and Haery, 1993) and the most frequently involved is the zygomatic maxillary complex (Lu *et al*., 2002). The progressive decrease in visual acuity is found in 50-90% of cases of FD and is due to bone proliferation and compression of the ONS (Katz and Nerad, 1998).

In the case of our patient, the compression of the optic canals gradually impaired the right optic nerve quite severely, producing its partial atrophy, due to bone proliferation in the FD, but also due to the compression of the optic chiasm by the large sphenoid mucocele. After evacuation of the sphenoid mucocele, vision in the left eye was fully recovered (Costea *et al*., 2015c). In FD, surgical treatment is controversial, ONS decompression being preventive or therapeutic (Abe *et al.*, 2002; Chen *et al*., 1997; Edgerton *et al*., 1985; Papay *et al.*, 1995).

Conclusions

Decreased visual acuity was the first symptom of FD, and also the patient's craniofacial deformity. The sphenoid mucocele associated with the FD were two entities diagnosed and treated surgically, the patient regaining complete visual acuity, only in the left eye (Costea *et al*., 2015c).

I.6.3. Researches regarding the use of mannitol in eye and brain surgery

Background

Mannitol has been used successfully in medical practice since 1940 (Smith *et al*., 1940). Later in 1960, the hypertonic solution was used to treat intracranial hypertension (IH) (Fardino, 2017), which was included in the list of essential medications by the WHO (WHO Model List of Essential Medicines, 2015).

We published an article regarding the use of mannitol in eye and brain surgery in collaboration with the Department of Neurosurgery of the "Prof.Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania and other collegues from other Departments of the "Grigore T.Popa" University of Medicine and Pharmacy, Iași, Romania and the data are presented as follows:

Turliuc MD, Cucu AI, Costachescu B, Tudor RM, Papacocea T, Bogdanici CM, Carauleanu A, Floria M, Tanase DM, **Costea CF**. The use of mannitol in neurosurgery and neuro-ophthalmology, *Cellulose Chemistry and Technology*, 2019a, 53(7-8): 625-633, (Impact factor in 2018 = 0.857).

Mannitol is used as a first-line therapy for the treatment and control of IH caused by cerebral edema from tumor pathology, strokes, subarachnoid haemorrhage and cranio-cerebral trauma (Papangelon *et.al*., 2009). It is used to lower Class I intracranial pressure (ICP) (Bratton *et al*., 2007) and in acute IH when transtentorial herniation occurs (Class III) (Bratton *et al*., 2007, Wakai *et al*., 2007). The physical and pharmokinetic properties of mannitol are shown in the Table I.12.

The optimal recommended dose of mannitol in the treatment of ICP is not precisely established or mentioned in the literature (Sorani *et al*., 2008). An intravenous dose of 0.15-0.20 g/kg for more than 30-60 minutes is considered safe for patients (Better *et al*., 1997, *EFSA, 2011, Flynn, 2007). The use of mannitol in ophthalmology was introduced by Contonnet (Shah and Maskati,1978; Contonnet, quoted by Duke-Elder,1904). Mannitol has been used as an ocular hypotensive as a hypertonic intravenous solution (Seeger and Lewis,1964). The ocular hypotensive effects of mannitol are useful in different ophthalmological diseases, such as acute angle closure glaucoma, chronic open angle glaucoma and different forms of secondary glaucoma or before intraocular surgery to reduce eye pressure (Seeger and Lewis,1964). It has the role of dissolving in the intravascular space, increasing the tonicity of the blood plasma, drawing water from the vitreous humor of the eye and lowering the ocular pressure (Tenny and Thorell, 2019; Mwsghali *et al*.,2019). The

recommended doses for ophthalmic use are from 0.25 g / kg to 2 g / kg administered intravenously 30 to 60 minutes, the effect occurring after 5-10 minutes and lasting about 6 hours (Tenny and Thorell, 2019; Mwsghali *et al*.,2019).

Administration before cataract surgery aims to increase the depth of the anterior chamber by decreasing the volume of the vitreous (O'Keeffe and Nabil,1983).

Table I.12. Mannitol and different concentrations of hypertonic saline (Freeman and Welbourne, 2018)

Solution	Sodium concentration (mmol L^{-1})	Osmolarity (mOsm L^{-1})	Equiosmolar dose mL (275 mOsm)	Dose (mL kg^{-1}) for 80 kg person
NaCl 0.9%	154	308	892	11
Ringer's lactate	130	275	1000	12.5
Saline 1.7%	291	582	472	5.9
Saline 3%	513	1027	268	3.4
Saline 5%	856	1711	161	2
Saline 7.5%	1283	2566	107	1.3
Saline 10%	1712	3424	80	1
Saline 30%	5000	10.000	27.5	0.34
Mannitol 10% (1 g mL^{-1})		549	502	6.3
Mannitol 20% (2 g mL^{-1})		1098	251	3.1

Smith and Drance in 1962 studied the reduction of intraocular pressure in normotensive and glaucomatous eyes along with blood osmolarity using mannitol solution and the results were the following: in normotensive eyes, the reduction of ocular tension was achieved in a percentage of 48% (average fall about 8 mm of Hg) and 52% in glaucomatous eyes with the return of intraocular pressure to its initial value between 2½ and 4½ hours from the time of the initial reading (Shah and Maskati,1978; Smith and Drance, 1962).

Regarding the side effects produced by mannitol, there are multiple of which we mention rebound phenomenon, with increasing ICP (Davis and Lucatorta, 1994, Shawkat *et al*., 2012, Troupp *et al*., 1971), hydroelectrolytic imbalances (hyperkalemia) (Preston *et al.*, 1998, Kaye and Grogono Reas), severe dehydration, progressive hyperosmolarity (Kaneda *et al.*, 2010), hypersensitivity reactions or cardiopulmonary edema (Davis and Lucatorto, 1994, Troupp *et al*., 1971) and acute renal failure (Nomani *et al.*, 2014).

In conclusion, mannitol has been used for about a century to reduce cerebral edema and the treatment of intracranial hypertension but also as an ocular hypotensive, by reducing the volume of the vitreous humor of the eye (Turliuc *et al.*,2019a).

I.7. Disambiguating the clinical and surgical anatomy

I.7.1. Studies regarding neuroanatomical terminology

Background

The art of treating the patient is based on anatomy, without which this would not be possible (Turmezei, 2012; Turliuc *et al*., 2016a).

We must not forget that "nothing is medicine without anatomy" (Di Dio, 1999).

Since the time of ancient Rome, when the clothing of the Romans highlighted their social status, it had a great influence on the name of various neuro-anatomical structures and was the basis of modern anatomical terminology (Turmezei, 2012; Turliuc *et al.*, 2016b).

Searching to understand the neuroanatomical terminology and the anatomical structures encountered in neuroophthalmic practice, I published two articles in collaboration with my colleagues from the Neurosurgery Department of the Emergency Clinical Hospital "Prof.Dr. Nicolae Oblu" Iași, Romania and with those from the Anatomy Discipline of the "Grigore T.Popa "University of Medicine and Pharmacy Iași, Romania, in ISI Web of Science journals, in which I highlighted the analogy between neuroanatomical structures and objects from Roman houses but also with Roman clothing (Turliuc *et al.*, 2016a; 2016b).

In the first article presented, we aimed to identify the origin of eight Latin neuroanatomical terms (pulvinar, capsule, infundibulum, operculum, forceps, habenula, flocculus, falx) and the correlations with ancient objects found in Roman households.

In the second article we identified possible analogies between neuroanatomical terminology and the clothing of ancient Romans. All these researches data are presented below:

Turliuc D, Turliuc S, Cucu A, Dumitrescu GF, Carauleanu A, Buzduga C, Tamas C, Sava A, **Costea CF**. A review of analogies between some neuroanatomical terms and Roman household objects. *Annals of Anatomy*, 2016 a, 204:127-133. IF = 1.864.

Turliuc DM, Turliuc S, Cucu AI, Sava A, Dumitrescu GF, Carauleanu A, Buzduga C, Trandafir D, **Costea CF**. An unwritten anatomy lesson: the influence of Roman clothing on neuroanatomical terminology: in memoriam Albert L. Rhoton, Jr. (1932–2016). *Clinical Anatomy*, 2016 b, 29:680-690. IF = 1.824.

I.7.1.1. Analogies between neuroanatomical structures and Roman furniture

Pulvinar **-** In ancient Rome, the pulvinar could be identified in all the villas, which was an armchair with many pillows called "pulvini", which took the form of an "empty throne" or a cushioned sofa where the Romans placed statues of their deities, during a conciliation ceremony with their gods "Lectisternium" (Smith, 1859). The pulvinar was a special "royal enclosure", where the Roman emperor sat on his imperial throne watching the circus or arena. The Roman emperor on the pulvinar looked like a godlike creature to the people (Pearson, 2013). Due to the anatomical resemblance to this ancient furniture, the caudal nucleus of the thalamus looked like an armchair, being called pulvinar in neuro-anatomical terminology (Federative Committee on Anatomical Terminology - FCAT,1998).

Between its arms there is no Roman deity, but the pineal gland. However, the French philosopher René Descartes (1596–1650) in the 17th century, asserted for pineal gland the role of a deity because he assigned it as the seat of intellect and soul (Figure I.111, A and B) (Turliuc *et al.*, 2016 a). The famous German professor of anatomy Karl Friedrich Burdach (1776–1847) was the first to use the name thalami pulvinar for this neuroanatomical structure (Poggi and Bossi, 1994; Meyer, 1970).

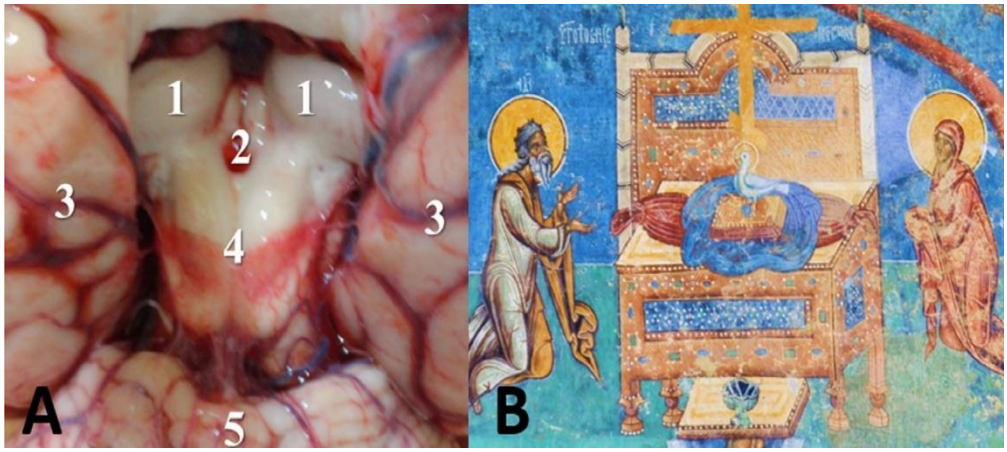

Figure I.111 - (A) Posterior view of the pineal region with: (1) pulvinar nuclei, (2) pineal gland, (3) medial view of occipital lobes, (4) tectal plate, (5) cerebellum. (B) Hetoimasia (Greek preparation), equivalent of the Roman empty throne (pulvinar) (or Throne of the Second Coming of Christ), used in Byzantine iconography on exterior picture of the western wall of Voronet Monastery, Romania, 1535 (detail) (Turliuc *et al*., 2016a).

Capsula interna **-** Going back to the ancient Roman villa, inside it there was a *capsa*, which was used as a repository or a chest or a *capsula*, which meant "small box or enclosure" (Diab, 1999). *Capsa* was the box was often placed next to statues dressed in togs, for holding

books. It was made of wood and had a cylindrical shape (Smith, 1959). In neuroanatomical terminology, FCAT (1998), the name *capsa* was used for a structure that designated a wrapper around another (Federative Committee on Anatomical Terminology - FCAT, 1998). Thus, there is *capsula externa* (external capsule), *capsula interna* (internal capsule), and *capsula extrema* (extreme capsule), which are white matter structures enclosing deep brain nuclei which appear to be seated in a box (capsule) (Figure I.112,A and B) (Turliuc *et al*., 2016a). As a neuroanatomical structure, Andreas Vesalius (1514-1564) was the first to illustrate the internal capsule. In his book "De humani corporis fabrica" ("On the Fabric of the Human Body") (1543), he mentioned in the Seventh Book that the letter E meant "the shining white matter" (Vesalius *et al*., 1543 / 2009), denominating what we now call "internal capsule". Thomas Willis (1621–1675), a renowned English anatomist, called this nervous structure "corporis striati limbus posterior" (Vieussens, 1775).

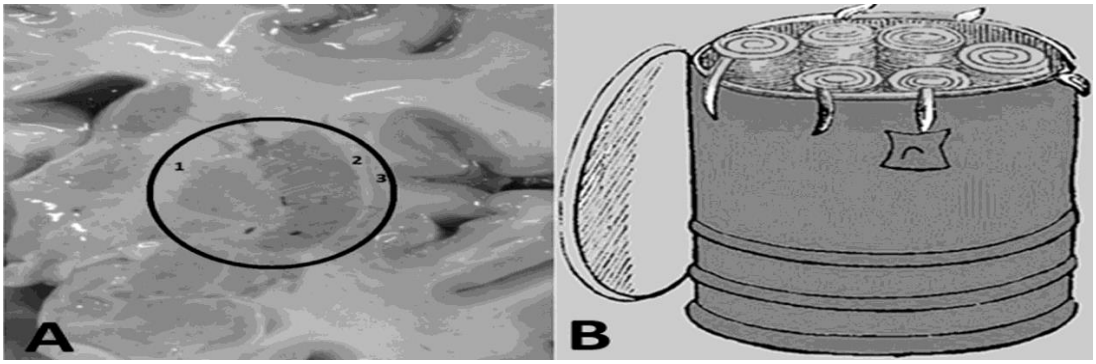

Figure I.112 – (A) Coronal section of brain to show (1) internal capsule, (2) external capsule and (3) extreme capsule. (B) An open capsa with six rolls of books in it-from a painting at Pompeii (adapted from Smith, W., 1859. A Dictionary of Greek and Roman Antiquities. Little Brown and Company, Boston) (Turliuc *et al*., 2016a).

I.7.1.2. Identification of some tools shapes in the morphology of neuroanatomical structures

Infundibulum **-** was an object in Roman households that looked like a funnel used to transfer liquids from vessels (Turliuc *et al*., 2016 a). The funnel-shaped neuroanatomical structure connected the basic pituitary gland of the brain and was called the infundibulum (Federative Committee on Anatomical Terminology - FCAT, 1998). This terminology was used by Rufus of Ephesus (80 - 150 AD), an ancient doctor, anatomist, and lexicographer (Diab, 1999) and by Galen of Pergamon (129 to c. 200 AD), a famous Greek philosopher and doctor of the Roman Empire (Swanson, 2014). Ruphus of Ephesus described this anatomical structure in his book "On the Names of Parts of the Human Body", the first one using the term infundibulum to describe it, due to its resemblance to the shape of a funnel (Diab, 1999).

Galen considered the infundibulum a neuroanatomical structure that has the function of a "funnel" for waste products from the ventricles to the pituitary gland (Swanson, 2014). He believed that the infundibulum filtered waste products that passed through the perforations of the palate into the mouth as mucus, pituitary or phlegm. He described the infundibulum as a cavity that extended from the pituitary gland (Swanson, 2014).

Operculum **-** represents in ancient Roman houses a little lid that covered jars and pots for cooking (White, 1975). In neuro-anatomical terminology, the frontal, temporal and parietal operculum are mentioned. This terminology has been adopted in neuroanatomy and has been found to refer to the parietal operculum, frontal operculum, and temporal operculum (Federative Committee on Anatomical Terminology - FCAT, 1998). Johann Christian Reil was the first anatomist to find during brain dissections the structure that covers the Sylvian fissure and looks like a roof. In 1809 he named it "das Dach der Sylvischen Grube" (Reil, 1809b) (roof of the Sylvian pit) which corresponds to what was later called the parietal and frontal operculum. In 1822, Burdach used the term "der Klappdeckel" in his book, regarding it as a head covering a pot (Burdach,1822).

In conclusion, ancient scholars discovered various anatomical structures in the brain that they named after various objects in Roman households, due to their similarity and shape. (Turliuc *et al.*,2016a).

I.7.1.3. Analogies between neuroanatomical structures and Roman clothing

Fimbria **-** Married Roman women (matrons) wore an adornment around the neck called segmentum - a sort of ribbon like a necklace, which could be embroidered with fringing called fimbriae (fringes) (Latin sg. *fimbria*, pl. *fimbriae*) (Adam *et al*., 1842). Other pieces of clothing for Roman women with edges made from the bare warp threads of the loom were also called “fimbriae” (Turliuc *et al.*, 2016b). This adornment was worn also by Roman men, but is was characteristic for woman clothing (Smith, 1853). In the ancient Rome homosexuals and effeminate men often used “fimbriae” in their attire. As it is mentioned in the literature even Julius Caesar, was described wearing fringed clothing (Hyrtl, 1880; Suetonius, 1913; Richlin, 1993). Anatomists were inspired by the Roman clothing “fimbriae” called the fringe of tissue from the uterine tubes due to their similarity (Turliuc *et al.,* 2016b). Herophilus of Alexandria, Rufus of Ephesus, and Eudemus first described the uterine fimbrial extremity (Buck and Stedman, 1914). Gabrielle Fallopius (1523–1562) correctly described this anatomical structure and called it the Fallopian tube (Macchi *et al.,* 2014). He was the first to introduce this term into anatomical terminology (Herrlinger and Feine, 1964). The prominent band of fringed white matter along the medial edge of the hippocampus, fimbriae

hippocampi (Hayman *et al.*, 1998) was described much later because its positioning within the brain made it very sensitive to putrefaction. The anatomists of those times lacked knowledge of how to approach and preserve it (Turliuc *et al.*, 2016b).

Cingulum **-** In Roman antiquity, the cingulum was a belt worn by churchmen (Lebby, 2013) or a cord made of wool that a bride wore over her frock. The wool was woven and represented the bond between husband and wife (Sebesta and Bonfante, 2001). In TA (FCAT, 1998), the denomination "cingulum" was given to a gyrus surrounding most of the corpus callosum like a belt (Turliuc *et al.,* 2016b). In 1809 the German anatomist Johann Christian Reil described "the girus cinguli" for the first time in the history of TA, together with other association tracts. He named it "tenia tecta" ("Bedeckten Baender" – covered bands). In 1822, Karl Friedrich Burdach named it cingulum owing to its likeness to the Roman belt (Burdach, 1822).

Lemniscus **-** The lemniscus was another piece of clothing used by the Romans (Latin: *lemniscus*-band). It was a band that women used, together with brooches, to fasten their clothing (Calza and Lugli, 1941) or as a head ornament (Smith, 1875). These bands (Latin pl. lemnisci) were also attached by woman to crowns or tiaras, hanging down from one's neck or back (Figure I.113, A-C) (Smith, 1875). The Swiss anatomist Albrecht von Haller, in 1765, mentioned that the white matter band was called lemniscus (Rasmussen and Peyton, 1948). In 1809, the famous German anatomist Johann Christian Reil was the first to study the path of this strip of white matter from the midbrain and pons, introducing the name lemniscus into neuroanatomy. In honor of Reil, the French anatomist Louis Pierre Gratiolet (1815–1865) called it "le riban de Reil" (Hyrtl, 1880).

Figure I.113 - (A) A lateral view of brainstem (lateral view) with medial lemniscus (blue) and lateral lemniscus (yellow) (Henry Gray, Anatomy of the Human Body, 1918). (B) The Augustus Emperor Cameo located in the centre of The Cross of Lothair wearing the lemniscus over the neck (Aachen Cathedral Treasury, early first century). (C) Romanian Byzantine iconography represented an angel with lemniscus around the head symbolising obedient listening to God's voice (public domain). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.] (Turliuc *et al.,* 2016b).

In 1867, the Austro-German anatomist Theodor Hermann Meynert suggested that the lumbar medial tract was the sensory tract (Boivie, 1971). Meynert was the first to differentiate the two parts of the upper (medial) and lower (lateral) lemniscus (Meyer, 1971).

Corona Radiata **-** In ancient Rome, corona (Latin corona - crown) was a circular ornament made of flowers, metal or leaves. It was placed around the neck or on one's head as a token of reward for talent, conquest or war, military success, civil merit or victory. Corona radiata was the crown offered to deities and to deified heroes and it was sometimes worn by kings as a sign of their divinity (Figure I.114, A-C) (Smith, 1853).

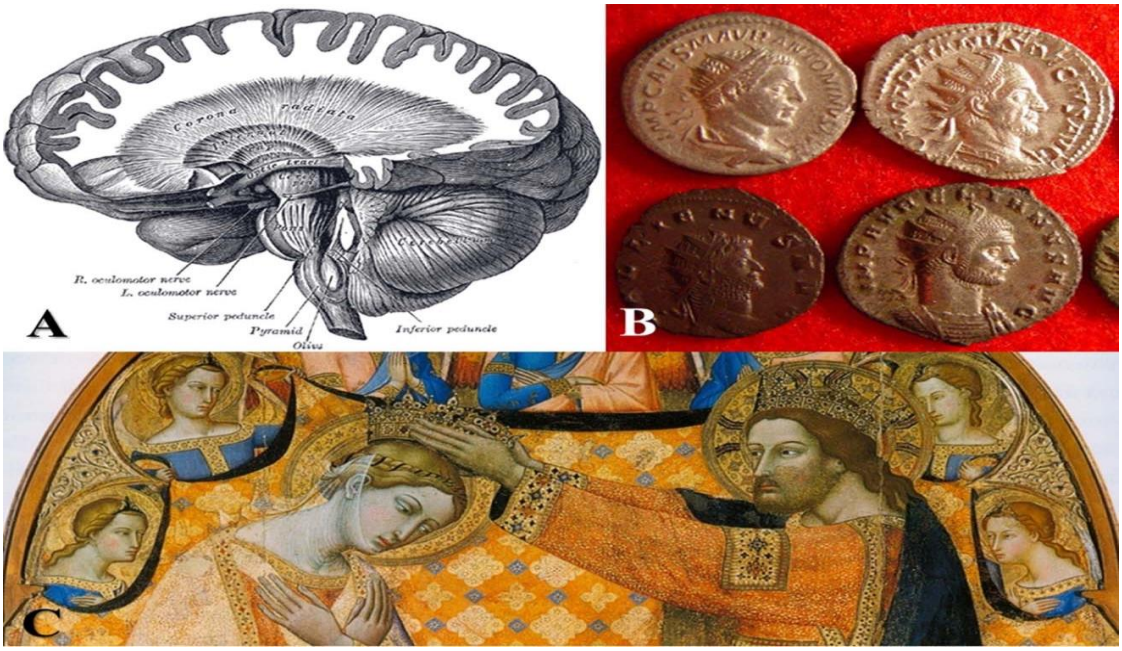

Figure I.114 - (A) Corona radiata (Henry Gray, Anatomy of the Human Body, 1918).
(B) Coins from Ancient Roman period, representing Roman Emperors with radiate crowns.
(C) God crowning Virgin Mary as Queen of Heaven, placing on her head the radiated crown (Jacopo di Mino del Pellicciaio, Coronation of Virgin-detail, 1340 - 1350) (public domain).
[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
(Turliuc *et al.*, 2016b).

The term Corona radiata (Stabkranz) was introduced by the German anatomist Johann Christian Reil in the neuro-anatomy terminology, describing the radiation of white matter tracts that reach deep into the brain (Schmahmann and Pandya, 2009) and in sagittal section that resemble the Corona Radiata worn by Roman emperors (Reil, 1812; Turliuc *et al.*, 2016b).

I.7.1.4. Crafts of ancient Rome and anatomical terminology – Systema Nervosum

Flocculus **-** The Latin word "flocculus" was used in Roman Antiquity to denominate a little tuft of wool (Venes, 2013) used by women to weave various clothing item (Turliuc *et*

al., 2016a). The neuro-anatomists were inspired by the image of a tuft of wool to name one of the lobes of the cerebellum as “flocculus cerebelli” (Federative Committee on Anatomical Terminology - FCAT, 1998) as from a macroscopic point of view these two elements shared similarities (Turliuc *et al.,* 2016a).The flocculus cerebelli, look like a tuft of wool and it is a little irregular lobule, in front of the biventral lobule, between the middle cerebellar peduncle and it is connected to the inferior medullary velum by its white core (Gray, 1918/2000).Michele Vincenzo Giacinto Malacarne (1744–1816), Italian surgeon and anatomist, founder of topographic anatomy recognized flocculus cerebellum (Burdach, 1822), being the first to describe the anatomy of the cerebellum in detail in his book "New exposure of the structure of the human brain", he used the term laminated flakes (Malacarne, 1776), introducing this term into the anatomical glossary (Parent, 2007).Félix Vicq d’Azyr identified the fourth lower cerebellar lobe and noticed that the surface was divided into several laminae just like sequencing strands of wool into a bale. He used the term flocculus, but could not identify what it was doing. The discovery of this small cerebellar lobe was made possible by brain fixation techniques during dissections, techniques developed by Dutch anatomist Frederik Ruysch (1638–1731) (Parent, 2007).

Habenula **-** At the level of the brain there is a fascinating anatomical structure called habenula (Federative Committee on Anatomical Terminology - FCAT, 1998). Its name comes from the Latin word "habena", which represents the reins the Romans used to restrain their horses (Smith, 1859). The habenular structure was first described by the Austro-German anatomist Theodor Hermann Meynert (1833–1892). He described in 1872 a small mass of gray matter at the posterior edge of the "stria medullaris" of the human brain, which he named "das Ganglion der Habenula". He was the one who identified the efferent main bundle of that area which he called “fasciculus retroflexus”, now called habenula-interpeduncular tract of Meynert (Jones, 1985). Meynert used the term habenula to refer to this anatomical structure and specified the synaptic function of the brainstem, pineal gland, and olfactory centers (Kahle and Frotscher, 2003).

Conclusions

Since ancient times, anatomists brought a great contribution to the understanding of the brain structure and spinal cord function. They discovered also new anatomical structures inspired by Roman civilization and its clothing, they created an anatomical terminology. The Romans and their clothing had such a great influence on European schools of anatomy, that we can say that they “dressed” the human body, making it immortal (Turliuc *et al*.,2016b). Neuroanatomical terminology has the origins in the analogies between the shapes of

anatomical structures in the brain as revealed by autopsies and the conformation of some household objects used by Romans (Turliuc *et al*.,2016a). The multitude of Latin words that are used in naming some neuroanatomical structures by analogies is a proof of neuroanatomists' fabulous imagination (Turliuc *et al.*, 2016a).

I.7.2. Icono-diagnosis - a medical - humanistic approach

Background

Icono-diagnosis represents the retrospective image-based diagnosis of pathologies on figurative arts. The term was introduced in 1983 by A. Pontius, a clinical professor of psychiatry at Harvard Medical School. He studied the Cook islands' prehistoric art, searching for the diagnosis of Crouzon's malformation (craniofacial dysostosis type I which is characterized by craniosynostosis, strabismus, hypertelorism, exophthalmia, "parrot-beaked nose", hypoplastic maxilla, a relative mandibular prognathism determining a mid-facial hypoplasia aspect, and a short upper lip (Symmers and Wallace, 1913; Pontius, 1983). However, earlier medical literature reported several example of this practise in sculptures and paintings with the translation from the images to a certain pathologic condition, just as two different languages (Craxi *et al.*, 2017). The anatomy of the human body an important played a role in art. Accurate observation of surface or external human body anatomy is essential in medicine and art. Speculations about artistic depictions of medical entities have been an ongoing pastime among physicians, yelding different suggestions and hypothesis. Portraits of an artist him/herself, or of others, may intentionally or unwillingly document a clinical condition or a genetic disorder in the subject. Such works of art provide a fascinating study for those with interests in the field (Pozzilli and Cappa, 2017). My researches regarding icono-diagnosis were published in two ISI ranked journals, one of them in Lancet Oncology with IF= 35.386.

Turliuc MD, Cucu AI, Perciaccante A, Tosolini G, De Luca S, Costachescu B, **Costea CF.** Hydrocephalus of King Charles II of Spain, the Bewitched King, *European Neurology*, 2019, 81(1-2): 76-78. IF=1.235.

Perciaccante A, Cucu AI, Coralli A, Turliuc MD, **Costea CF**, Bianucci R. History of Medicine Mid-19th century Chinese medical portraits depict late-stage female breast tumours, *Lancet Oncology*, 2019, 20(10): 1347-1348. IF=35.386.

I.7.2.1. Icono-diagnosis study on king Charles II of Spain's hydrocephalus

In 1685, the painter Juan Carreno de Miranda painted a portrait of King Charles II of Spain. He highlighted a person with a bumpy forehead and protruding jaw. Based on this

painting, we issued a clinical diagnosis hypothesis in order to establish an icono-diagnosis (Turliuc *et al*., 2019). King Charles II of Spain (1661-1700) (Figure I.115, a-c) suffered from a variety of illnesses during his lifetime (Bennassar, 2000, Gargantilla 2005, Turliuc *et al.*, 2019, Kamen, 1983) and was known as El Hechizado, who was mentally retarded and physically disabled and disfigured (Alvarez *et al*., 2009). Subsequent research has established that it came from a Spanish branch of the Habsburg family in which inbreeding was common (Alvarez *et al.*, 2009, Ceballos and Alvarez 2013, Turliuc *et al.*, 2019). Charles could not speak until the age of 4 years and he could not walk until the age of 8-10 years (Alvarez *et al.*, 2009, Littell, 1849), and "his mind, too, was a constant prey to a corroding melancholy, which appears to have been in a great measure produced by the most ignoble and womanish superstitions (Dunlop, 1834, Turliuc *et al.*, 2019). He was treated like a baby until he was 10 and left completely uneducated, for fear of overstraining a fragile child (Littel, 1849).

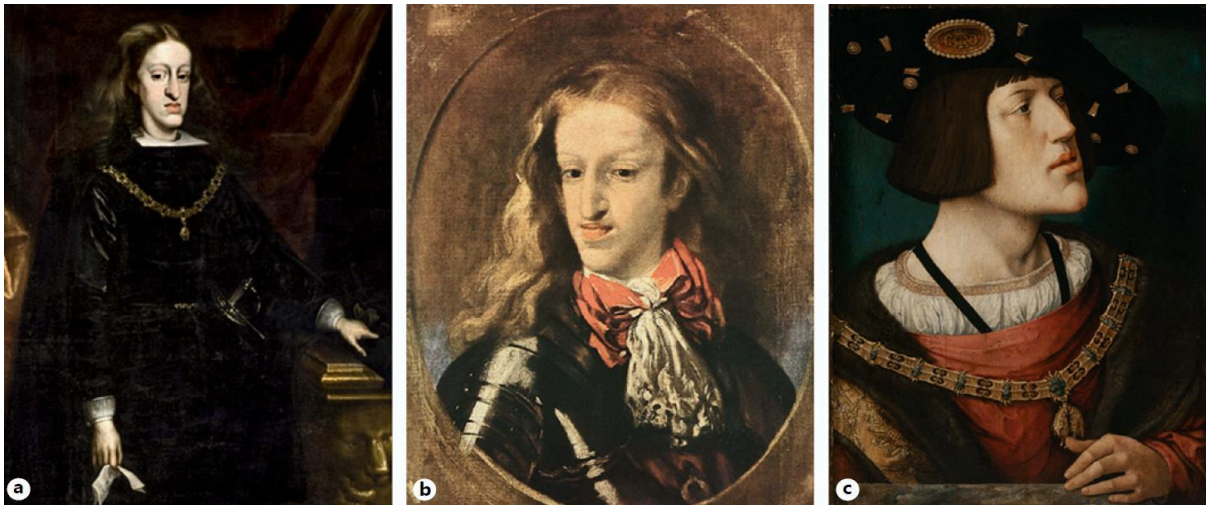

Figure I.115. Charles II of Spain, Juan Carreño de Miranda (c. 1685), Museum of Art History, Vienna (a). King Charles II, Claudio Coello (1675-1680), The Prado Museum, Madrid (b). Portrait of Charles V, Bernard van Orley (c.1515-1516), Museum of Fine Arts, Budapest (c) (public domain).

The American historians Will and Ariel Durant described Charles II as "short, lame, epileptic, senile and completely bald before 35, he was always on the verge of death, but repeatedly baffled Christendom by continuing to live" (Durant and Durant, 1963). He eventually died in Madrid at the age of 38. King Charles II of Spain has been found to suffer from many genetic diseases, as many authors have speculated, such as Fragile Syndrome (Navalon Ramon and Ferrando Lucas, 2006), Klinefer syndrome (Gargantilla 2005), pituitary hormone deficiency and renal tubular acidosis (Alvarez *et al.*, 2009), or male hermaphroditism with Fragile Syndrome (Garcia-Escudero Lopez *et al*., 2009). Regardless of

the diseases that this king was speculated to have suffered from, signs and symptoms such as macroencephaly, mental retardation and growth retardation as well as vomiting and epileptic seizures in his childhood were linked to the diagnosis of hydroencephaly (Turliuc *et al*., 2019).

Although there is no clear evidence that could support our etiological assumption related to Charles II's hydrocephalus, we may however argue that herpetic infection, which he was believed to have suffered from after his birth, may have caused it, as herpetic infections are known to cause hydrocephalus (Hayashi *et al.,* 1986, Takano *et al.,* 1995).

I.7.2.2. Icono-diagnosis study on Chinese medical portraits depicting the stage of a female with breast cancer

We reported one of the largest pictorials in the nineteenth century collection of breast tumors, painted by Kwan Kiu Cheong (Lam Qua) (1801-1860) (Yale University, Peter Parkers, Lam Qua paintings collection, Perciacante *et al.,* 2019). Dr. Peter Parker (1804-1888) was the founder of the first Western hospital in China, the Ophthalmic Hospital in Canton (Perciaccante *et al*., 2019; Chan *et al.*, 2011).

Lam Qua was the painter who painted the portraits of the patients admitted to this hospital (Perciaccante *et al*., 2019). Doctor Parker commissioned Kwan Kiu Cheong to paint preoperative portraits of ill people who had large neoplasia or other important abnormality and who came to his hospital for treatment. Among these at least 115 oil paintings (Gilman, 1986), there is an impressive collection of 80 paintings held by the Harvey Cushing / John Hay Whitney Medical Library at Yale University (Yale University. Peter Parker's Lam Qua paintings collection).

We analyzed 80 pictures and found that 37% (n = 11) were advanced breast cancers of some Chinese women. The macroscopic appearance of the lesions led us to conclude that the painter had highlighted Paget's disease of the breast (Karsakas, 2011).

Portrait no. 24 shows few signs specific of this type of cancer: eczema-like rash in the nipple area and adjacent areolar skin, ulceration and nipple inversion (Figure I.116 A and B) (Perciaccante *et al.,* 2019).

The entire picture collection gives us information about the epidemiology of breast cancer during this period in China (Perciaccante *et al.,* 2019).

Furthermore, the International Association of Cancer Registries reported that the most common neoplasia in Chinese women is breast neoplasia, with an incidence of 21.6 cases per 100.000 (Fan *et al*., 2014, Bray *et al.,* 2018).

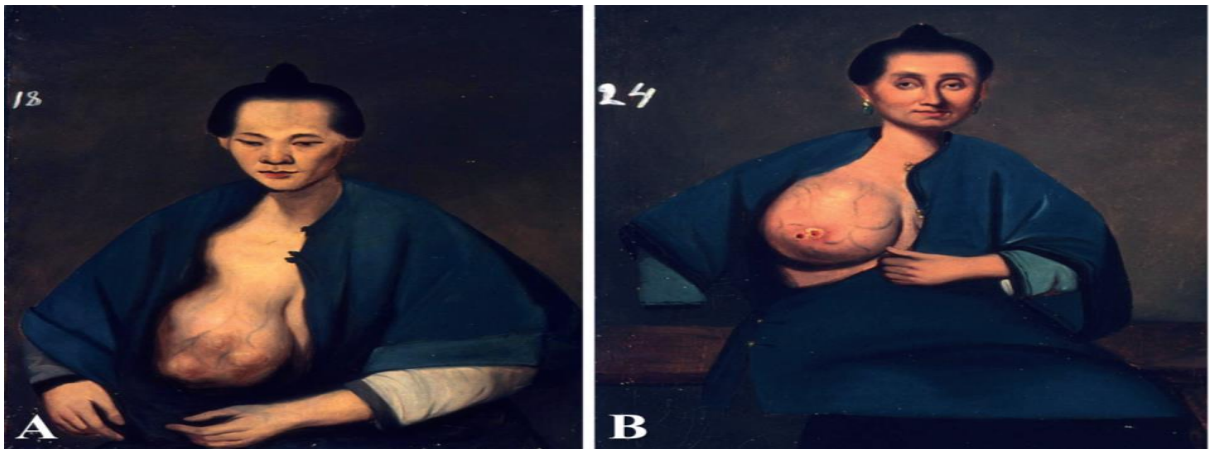

Figure I.116. Portrait number 18. Description: woman seated. A large, malignant tumor is evident on her right breast, (1830-1850) (A). Portrait number 24 shows a possible case of Paget's disease of the breast (1830-1850) (B), Yale University, Harvey Cushing/John Hay Whitney Medical Library (Perciaccante *et al*., 2019).From the appearance of the lesions, we concluded that the patients admitted to Dr. Parker's hospital were in an advanced stage of the disease, indicating the cultural and historical aspects of the mid-nineties in China (Kang 2012, Perciaccante *et al*., 2019).

Also, the Chinese National Central Cancer Registry demonstrated, in 2008 that breast neoplasia is the most common type of cancer among women in urban areas and the fourth most common in rural regions (IACR, Fan *et al*., 2014, Perciaccante *et al*., 2019).

SECTION II - FUTURE PLANS OF PROFESSIONAL, SCIENTIFIC AND ACADEMIC CAREER DEVELOPMENT

II.1. Development of the professional career

The professional experience gained during the 18 years of activity carried out in the "Prof.Dr.Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania, in the Second Ophthalmology Clinic, determines me to strive permanently for improvement. It is imperative to continue with the same intense efforts to become a better ophthalmologist tomorrow than today. This desideratum will be achieved by continuing enriching my theoretical and practical knowledge, by deepening my professional training alongside five main directions:1.Oncologic and inflammatory ophthalmology, 2.Ocular reconstruction, 3.Ophthalmology interdisciplinary approach, 4.Neuro-ophthalmology, 5. Neuro-anatomical terminology and icono-diagnosis as a medical-humanistic approach.

The treatment of oncological ocular diseases, which is carried out after setting the diagnosis, following complex and detailed investigations, a special contribution along with my clinical and surgical activity having the pathological anatomy laboratory within the "Prof.

Dr. Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania. In order to make the most precise therapeutic decisions, I propose to collaborate more with the doctors from the hospital's own laboratory and sometimes, when it is the case, with the consultation of other specialists from other university clinics. In most cases, the therapy of ocular diseases with oncological specificity is surgery, most of the times conducive to healing. During surgical interventions, operative steps are performed with the greatest possible precision to achieve better results and to heal the patient. In order to bring up my training in ocular surgery to a higher level, I propose to participate in sessions of scientific communications, congresses and/or exchanges of experience in clinics specialized in ophthalmology. Regarding the treatment of ophthalmologic diseases, I keep updated consulting international and national ophthalmology publications. During my 18 years of medical practice, I gained a rich experience that helps me to easily master the novelties that appear in the specialized literature. I propose to continue participating in scientific events and congresses organized by prestigious forums in the country and abroad. So far, I participated in 22 theoretical and practical postgraduate courses in ophthalmology organized in the country and abroad on the occasion of specialized congresses. I also attended 24 postgraduate courses in ophthalmic surgery. These helped me a lot in the formation and development of my professional career, which is why I will continue to participate in such courses by choosing the ones that will contribute to the development and improvement of my professional career. My participation in 46 international congresses as well as 82 national congresses, conferences and symposiums gave me the opportunity to be informed with an important volume of theoretical and practical knowledge in the field of ophthalmology and related fields. I participated as a member in 15 academic and professional societies with the perspective of extending the participation to other professional societies of international prestige. I have the opportunity to contribute to the publication in international journals of new articles in ophthalmology performing peer-reviews for 9 prestigious international journals. I will intensify the collaboration for highly complex surgical interventions related to the orbital tumors with neurosurgeons and other neurosurgeons from other Neurosurgery Clinics from the “Prof.Dr.Nicolae Oblu” Emergency Clinical Hospital of Iași, Romania and from the “St. Maria” Children's Hospital from Iași, Romania, with whom I have a very intense and complex collaboration. I had the opportunity to participate in complex surgeries, as a volunteer and at the request of the “St. Maria” Children's Hospital of Iași, for the surgical treatment of some particularly complex cases of orbital tumors in children patients. This convinces me that my professional training will have a good evolution with beneficial effects for patients with ophthalmic and related conditions.

II.2. Development of the scientific career. Research directions

The experience and the results achieved during the 15 years after finishing my doctoral studies, help me to continue the development of my scientific research. The beginning was particularly interesting with the topic of the doctoral thesis: "Considerations on various methods of treatment of malignant eyelids tumors", which was a significant challenge in the development of my research. On that occasion I realized that the field is vast and only through assiduous work you can accumulate valuable knowledge in ophthalmology during the four years I have been working on my doctoral thesis. The subject of the doctoral thesis as suggested by Prof. PhD MD Sergiu Buiuc, along with other proposed topics, was chosen further to the need of complete research in this direction of the methods of treatment of eyelids tumors. After obtaining the title of doctor in medical sciences, Ophthalmology specialty confirmed by the Ministry of Education and Research and Technology by Order No. 3443 / 12.03.2008 and after obtaining the Diploma of Doctor in medical sciences, Ophthalmology specialty with No. 1404 / 17.04.2008, I broadened my fields of research to five main directions: 1.Oncologic ophthalmology; 2.Eye surface and orbital reconstruction; 3.Ophthalmology interdisciplinary approach; 4. Neuro-ophthalmology; 5.Neuro-anatomical terminology and icono-diagnosis as a medical-humanistic approach.

• **Oncologic ophthalmology -** The "Beauty and the Beast" project is my main and immediate focus. I named it so because of the feeling I have when I beat cancer in this field. Nothing crowns my work more than restoring a patient's vision and eliminating a vital danger for his life. Of all ocular and periocular tumors, eyelid cancers have been and remain my main concern. I will also further research in eyelid tumors pathology associated with other systemic diseases. It is very important to recognize the systemic implications and to consider the genetic evaluation of patients who develop eyelid tumors within genetic syndromes. Most eyelid tumors have a genetic predisposition, such as Gorlin-Goltz syndrome. I will focus on the study of sebaceous carcinomas of the eyelids, myxomas and on rare anatomical and pathological aspects identified in malignant eyelid tumors, in the presence of systemic associations. Orbital tumors in children represent a different spectrum than those diagnosed in adults, the most common are of mesenchymal origin, the most common being rhabdomyosarcoma. Lymphoma also occurs in the same age group in children as rhabdomyosarcoma. Future research will consider clinical trials of these orbital tumors in children, surgical treatment combined with radiation therapy and chemotherapy depending on the stage of the disease. Anatomo-clinical research related to eye, periocular and orbital tumors will be carried out in collaboration with the Pathological Anatomy Laboratory of the

"Prof. Dr. Nicola Oblu" Emergency Clinical Hospital and the "St. Mary" Emergency Clinical Hospital for Children of Iași, Romania. This research in the field of ophthalmology-pediatrics will be carried out in collaboration with Plastic and Reparative Surgery, Neurosurgery and Oncology Clinics of the "St. Mary" Emergency Clinical Hospital for Children of Iași, Romania.

• **Eye surface and orbital reconstruction** - The "Window of the Eye" is the second project I intend to continue. The integrity of the ocular surface and the corneal transparency are necessary for the best visual acuity of the patient. Infections on the corneal surface and loss of corneal tissue due to burns or malignant or benign lesions are often a challenge for the ophthalmologist. In ocular surface diseases that are determined by the deficiency of limbic stem cells, the best therapeutic option is the transplantation of limbic stem cells. This results in the transparency of the cornea after severe injuries, such as burns to the eye surface. Transplantation of the amniotic membrane in ocular surface tissue loss as a result of excision of benign or malignant lesions, and transplantation of limbic stem cells in eye surface diseases for ocular surface reconstruction are another topics in which I will continue and develop my research. Oculo-orbital tumors and traumas that result in severe cases of the loss of the eyeball and prosthesis of the orbital cavity have been in my attention throughout my clinical and scientific work. I propose to continue the research on the morpho-functional and aesthetic recovery of the patient in order for his social reintegration and a better life quality of the adult and children patient. I will carry out the research regarding the amniotic membrane transplant for eye reconstruction, in collaboration with the Gynecology Clinic of the “Cuza Vodă” Clinical Hospital of Obstetrics-Gynecology of Iași, Romania. I will also consider different surgical techniques for reconstructing the ocular surface in cases of severe keratitis and after excision of extensive tumor lesions of the ocular surface, but also in cases of severe thermal or chemical ocular burns, which require transplantation of limbic stem cells and amniotic membrane.

• **Interdisciplinary ophthalmology** - Eye diseases such as age-related macular degeneration, diabetic retinopathy, hypertensive retinopathy and thyroid ophthalmopathy require interdisciplinary collaboration to identify the endogenous and exogenous factors that determine the etiology of these diseases. Collaborating with other related disciplines to diagnose and treat these patients is also my plan for the future. I also rely on an intense international collaboration project in order to establish pilot and collaborative translational and clinical studies, development of communication pathways, to obtain centralized support for research design, epidemiology, biostatistics and clinical research ethics with other clinics,

regulatory knowledge and research education, training and career development. The interdisciplinary collaborations will take into account the development of research on age-related macular degeneration and microbiota, which will be carried out in collaboration with the Internal Medicine Clinic of the County "St. Spiridon" Emergency Clinical Hospital of Iași, Romania Also, my concern in the future will include exophthalmos in endocrinological diseases, in collaboration with the Endocrinology Clinic of the "Sf. Spiridon" County Emergency Clinical Hospital in Iași, Romania. I will continue my anatomical-clinical research related to thyroid pathology with the Pathological Anatomy Laboratory of the "Sf. Spiridon" County Emergency Clinical Hospital in Iași, Romania.

• **Neuro-ophthalmology -** My "Eyes of the Mind" project is about optical coherence tomography (OCT), which is a method of investigating axonal / neuronal integrity and the progression of eye and neurological diseases that affect visual pathways. Spectral-domain OCT (SD-OCT) is the technology that can quantify changes in the retinal nerve fiber layer (RNFL) macula volume and the vascular structure of the eye in neurodegenerative pathologies. In diseases such as optic atrophy, ischemic optic neuropathy and optic neuritis, provides information on the analysis of irreversible neuronal loss. Posterior eye-segment diagnosis using OCT also provides information on the progression of Alzheimer's and Parkinson's diseases. In the future, I intend to investigate the contributions of these investigations to the progression of neuro-ophthalmic diseases mentioned above. I will carry out research related to neuro-ophthalmic diseases in collaboration with the 1st and 2nd Neurology Clinic of the Emergency "Prof.Dr.Nicolae Oblu" Clinical Hospital of Iași, Romania. Also, brain tumors pathology with implications on the visual system is a present and future concern. I will continue the research related to this research direction together with colleagues from the Second Clinic of Neurosurgery of the "Prof.Dr.Nicolae Oblu" Emergency Clinical Hospital of Iași, Romania. Also, the vascular cerebral diseases like carotid-cavernous fistulas, are in my attention, now and in the future, to develop research related to the impact and visual recovery of these patients with severe exophthalmos caused by carotid-cavernous fistulas.This line of research is developed currently and in the future in collaboration with the Laboratory of Radiology and Medical Imaging of the Second Clinic of Neurosurgery of the "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital Iași, Romania.

• **Icono-diagnosis and neuroanatomical terminology -** This new method, icono-diagnosis, uses pattern detection based on the known facts of patho-physiology of body functioning. This method has so far been found to be fruitful in detecting malfunction or malformation of the human body using specific deviations represented pictorially in hundreds

of samples, perpetuated over hundreds of years. What is the relevance of applying this new method of icono-diagnosis to the art? There are two sets of reasons to do so: a heuristic practical one providing a workable theory and specific directions for future research; and a heuristic theoretical reason helping to conceptualize factors that contribute to the emergence of the human capacity to create representational art beginning with depicting the human body in a puzzling variety of shapes.Regarding the icono-diagnosis and neuroanatomical terminology, I intend to continue and develop collaborations with the Department of Anatomy of the "Grigore T. Popa" University of Medicine and Pharmacy of Iași, paving the way for combining the study of clinical diagnosis with the field of medical art and neuro-anatomical terminology.

II.3. Development of academic career

The beginning of my academic activity started in 2005, when via competitive examination I occupied the position of Junior Assistant Professor in Ophthalmology at the “Grigore T.Popa” University of Medicine and Pharmacy of Iași, Romania. This important step in the beginning of my academic career was an important challenge, because it gave me the opportunity to verify my theoretical and practical knowledge acquired in college and in the intern ship year held at the "Sf. Ioan" Emergency Hospital in Iași. This teaching position was an important opportunity for me to assert myself on multiple levels, because the third year students, the Faculty of Dentistry of “Grigore T.Popa” University of Medicine and Pharmacy of Iași, Romania, for which I work, came both from our country and from other English and French speaking countries. This opportunity gave me the opportunity to communicate in French and English, which for me was not a difficulty, because I am an advanced user of French and English languages. Over the years, I was promoted via competitive examination as follows: assistant professor (2008), lecturer (2018), associate professor (2020). Each stage between these promotions was dominated by intense work, consistently respecting the proposed development directions. Thus, in my academic career I acted with a lot of perseverance in the meticulous preparation of teaching materials for teaching practical works and courses in Romanian, English and French for the ophthalmology discipline of “Grigore T.Popa” University of Medicine and Pharmacy of Iași, Romania.I tried to choose a way to present the curricular notions as valuable as possible in terms of scientific content and interactively to attract students to the discipline of ophthalmology and to make them go home at the end of each lecture with a minimum of knowledge retained without much effort. Thus, we created a pleasant environment for participating in practical work and courses. The practical works occupied an important percentage in the application of

theoretical knowledge from courses. This activity is very important to me, I find great satisfaction in seeing that students acquired the necessary knowledge. The clinical case presentations in the practical works had an important role in the knowledge of the most frequent ophthalmological diseases. The presentation of the power-point courses allowed the possibility of structuring, synthesizing and exemplifying through images the most significant ocular diseases. I continue on this path by constantly updating the content of the courses for students and resident ophthalmologists with the news published in the ophthalmology literature, as well as with examples from my clinical activity. This approach to presenting the courses captivates the audience, which showed an interest in the knowledge, asked questions for additional clarifications given to students and resident doctors at the end of each ophthalmology course.

My academic activity also has a publishing component, authoring and co-authoring books on various topics to help students, resident, specialists and senior ophthalmologists, in deepening the theoretical and practical knowledge of surgery, in the vast field of ophthalmology. Thus, the book "Guide to ophthalmic pathology. Eyelid and conjunctiva diseases", Romanian edition published by the Publishing House of the Academy of Sciences of the Republic of Moldova in Chișinau (2015), 428 pages, includes an in-depth presentation of the diseases the eye. For French-speaking students who are quite numerous at our university, I have also published this book in French through Universa Publishing House, 9230 Wetteren, Belgium, (2015), 442 pages.

A very important component of the academic activity is the theoretical and practical training of the resident doctors in the specialty of ophthalmology and in related disciplines who carry out practical internships in ophthalmology. I developed theoretical courses according to the ophthalmology curriculum in a power-point presentations with a higher scientific level, so as to help them to enrich their knowledge according to the stage they go through in becoming specialists at the end of the internship. Practical training in the hospital at the patient's bedside gives them skills in approaching the diagnosis and treatment of each patient. The clinical case presentations made regularly by each resident doctor must be very well prepared so as to include the most useful and well-documented notions regarding the case in question. Completing the clinical case presentations with discussions in which each resident doctor participates with their own opinions and clarifying questions, makes this activity achieve its purpose for which they were organized. One component that I want to develop in the future in my academic activity is guiding doctoral students in ophthalmological research during doctoral studies and publishing research results in

prestigious journals abroad. The publication of scientific articles in journals, as well as scientific communications on the occasion of congresses and conferences in the field of ophthalmology or other medical fields, offers the opportunity to dissipate the results of research conducted during the elaboration of the doctoral thesis.

I will orient the doctoral students towards the choice of research topics for the elaboration of the doctoral thesis in ophthalmic medical sciences towards the deepening of some aspects that until now have not been sufficiently studied.

The results must contribute to the improvement of the medical act. My participation as an elected member of the Board of the Department of Surgery II, since 2016 offers me opportunities in terms of leading a structure in the organization of the "Grigore T.Popa" University of Medicine and Pharmacy of Iași, Romania. The Department of Surgery II consists of clinics specializing in neurosurgery, ophthalmology, ENT, radiology, plastic surgery, emergency medicine.

This activity is beneficial for completing the knowledge in the managerial field, which determines me to orient in a future stage my participation to other higher structures within the "Grigore T.Popa" University of Medicine and Pharmacy of Iași, Romania.

Also, my participation in the doctoral admission commissions of the disciplines of ophthalmology and neurosurgery, the commissions for the promotion teachers in the Ophthalmology Discipline and other related disciplines and the specialized and senior examination commissions in ophthalmology, make me available for participation in the promotion and validation of ophthalmologists and other related specialists, in our university or other universities where I may be requested, contributing to the development of my academic activity.

II.4. Conclusions

In conclusion, the habilitation thesis is the presentation of my scientific, professional and academic activity representing the starting point in the previously mentioned projects, which I will develop in the future.

The clinical and scientific experience I have gained so far, provides me with a solid foundation for the research directions mentioned above, which I will deepen and which will facilitate my future interdisciplinary collaborations, contributing to the efficiency of my clinical, teaching, scientific and academic studies in ophthalmology.

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