GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

HABILITATION THESIS

INTERNAL MEDICINE AT THE INTERFACE BETWEEN SPECIALITIES

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"A învăța pe alții, înseamnă a te învăța mai întâi pe tine."

(François de la Rochefoucauld)

CONTENTS

Abbreviations list

ACE2-R = angiotensin-converting enzyme 2 cellular receptor

AGEP = Acute Generalized Exanthematous Pustulosis

AGEFs = advanced glycation end products

AI = artificial intelligence

AIX = augmentation index

AS = ankylosing spondylitis

ASI = arterial stiffness index

AUC = area under curve

AuNPs = gold nanomaterials

BaPWV = brachial-ankle PWV

BMI = increased body mass index

BOP = bleeding on probing

CAL = clinical attachment level

CAT = catalase

CAVI = cardio-ankle vascular index

CFU = Colony Forming Units

CfPWV = carotid-femoral PWV

CoV = coronavirus

CRC = Colorectal cancer

CR = cardiovascular rehabilitation

CT = Computer tomography

DAA = Direct-acting antiviral

DM = Diabetes mellitus

DOX = doxorubicin

DRESS = Drug reaction with eosinophilia and systemic symptoms

ECDC= European Centre for Disease Prevention and Control

ED = endothelial dysfunction

ESBLs = extended-spectrum β-lactamases

EUCAST = Antimicrobial Susceptibility Testing

FDG = fluorodeoxyglucose

FDG-PET = positron emission tomography

FMD = flow-mediated dilation

FPG = fasting plasma glucose

FTIR = infrared spectroscopy

GCF = gingival crevicular fluid

HCV = hepatitis C virus

HCC = hepatocellular carcinoma

HF = heart failure

HTN = arterial hypertension

LCR = lymphocyte-to-C-reactive protein ratio

MDA = oxidative stress

MDA = malondialdehyde

MDR = multi drug resistance

MERS CoV = Middle East respiratory syndrome coronavirus

MPV = mean platelet volume ratio

NAFDL = Non-alcoholic fatty liver disease

NASH = non-alcoholic steatohepatitis

NLR = The neutrophil to lymphocyte ratio

NLRP3 = NLR family pyrin domain containing 3

NMD = nitroglycerin mediated dilation

NO = nitric oxid

OLP = oral lichen planus

OR = Odds Ratio

PAD = peripheral artery disease

PD = probing depth

PEG = Polyethylene glycol

PLR = platelet to lymphocyte ratio

PNLPA3 = patatin like phospholipase domain containing 3

PWV= puls wave velocityQoL = quality of life

ROS = reactive oxygen species

RT-PCR = Reverse transcription polymerase chain reaction

SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

SCV = sustained virologic response

SD = standard deviation

SOD = superoxide dismutase

Sp As = Spondyloarthropathies

SVR = sustained virologic response

TAT peptide = cell-penetrating peptide

TAT = trans activator of transcription

TEB = thoracic electrical bioimpedance

TMPRSS2 = transmembrane srine protease 2

UTIs= Urinary tract infections

WHO = World Health Organization

Abstract

Habilitation Thesis with the Title *"Internal Medicine at the Interface of Specialties"* is elaborated according to the recommendations of the National Council for Titles, Diplomas, and University Certificates Certification (CNATDCU), and the methodology of the Doctoral School of "Grigore T. Popa" University in Iași. The habilitation thesis consists of three sections as follows:

In the first section, I reviewed my academic journey, starting in 1991 as an assistant professor at the Department of Medical Semiology within the Clinical Hospital C.F. Iași, then becoming a senior lecturer and associate professor from 2020 to the present, both in the academic and clinical spheres. My scientific activity began with my enrollment in a doctoral program, which I completed in 2002 with the thesis "Current Antibiotic Therapy in the Treatment of Bacterial Pneumonia" within the field of Infectious Diseases. My interests in this field and in the interrelations of internal medicine with other specialties continued during my postdoctoral period, resulting in the publication of 25 ISI papers, 13 as the main author, 4 ISI abstracts, and 4 proceedings, along with 29 BDI articles. Recognizing the quality of these works, 3 of them were awarded by UEFSCDI. Additionally, my Hirsch Index is 9, and the cumulative impact factor of my main-authored ISI articles is 29.03.

My scientific activity is structured around 4 research directions as follows:

Chapter 1 presents the results of my work in the field of infectious diseases. Subchapter I.1.2.1 focuses on Covid-19 pathology, which I encountered during the pandemic years**.** In 2020, WHO announced the official name of the new virus identified in Wuhan, China, as "SARS-CoV-2," causing acute respiratory syndrome. It belongs to the sub-family of Coronaviruses: 229E, NL63, OC43, HKU1, SARS-CoV, MERSCoV. I conducted an epidemiological retrospective study of SARS-CoV-2 infection, identifying its manifestations in Moldova. The evolution of these manifestations has been consistent with the patient's general condition up to that point (subchapter I.1.2.1.3). Hypoxia, systemic stress involving inflammatory factors, thrombosis, arterial stiffness, inflammatory response, and multi-organ failure are obvious outcomes of the virus based on current data. Concerns arise regarding arterial remodeling in patients with existing vascular disease and the potential development of persistent, chronic COVID-19 vasculopathy (subchapter I.1.2.1.1). Polymorphic skin and mucosal lesions associated with SARS-CoV-2 are not solely due to viral etiology. The variety of clinical aspects may arise from pathogenic differences, host reactivity, co-infections, and viral-induced vasculitis and thrombotic vasculopathy (subchapter I.1.2.1.2).

Continuing within the domain of infectious diseases, alongside SARS-CoV-2 infection, this chapter reviews the interrelations between biochemical changes and antibiotic treatment, particularly regarding oral cavity microbiota (subchapter I.1.2.2.1).

Chapter 2 refers to research in the field of metabolic syndrome. Metabolic syndrome encompasses metabolic abnormalities like hypertension, central obesity, insulin resistance, and atherogenic dyslipidemia. Metabolic-associated fatty liver disease is diagnosed based on hepatic steatosis findings, type 2 diabetes, overweight, obesity, or hyperlipidemia. The connection between diabetes and non-alcoholic fatty liver disease (NAFDL) is known. NAFDL covers a spectrum of disorders including hepatic steatosis, inflammation, cirrhosis, fibrosis, and hepatocarcinoma (subchapter I.2.2.1.2). Cardiovascular disease is a leading cause of death among people with diabetes, especially type 2 diabetes (subchapter I. 2.2.2). The role played by novel inflammatory markers in assessement of peripheral artery disease along with haematological characteristics of coronary patients with metabolic comorbidities are presenting in this subchapiter. Hyperglycemia in diabetes is implicated in altering oral microbial flora, and also produce

imbalance of inflammatory cytokines profile in gingival crevicular fluid (subchapter I.2.2.3). Other oral pathologies are presented in subchapiter I.2.2.4.

Chapter 3 outlines my research in immune-mediated rheumatic disorders and their systemic implications. Some spondylarthritis cases don't respond to anti-TNF agents or experience drug toxicity due to anti-drug antibodies. My study assessed the relationship between immunogenicity, drug levels, and clinical efficacy of TNF inhibitors in ankylosing spondylitis. Atopic disorders such as rhinitis, atopic dermatitis, and asthma may arise in spondyloarthropathy patients on biological therapy. Atopic patients may need more frequent biologic switching among biologigs to control their active disease (subchapter I.3.2.1). Data about lipoprotein changes and their link with cardiovascular disease and atherosclerosis in systemic sclerosis (SSc) are still challenging. Patients with SSc are at risk to develop abnormal lipid profile (with low serum HDL-cholesterol and high LDL-cholesterol levels, high triglycerides, and total cholesterol), particularly those with a diffuse cutaneous SSc, those with active and severe disease, and specific autoantibodies (subchapter I.3.2.2). Chronic inflammatory rheumatic disorders (IRD) such as rheumatoid arthritis (RA), ankylosis spondylitis (AS) and other spondylarthropathies (SpA) are broadly characterized by a significant economic and illness burden, mainly related to disease activity, severity, as well as disability and impaired quality of life. TMJ arthritis significantly correlates with disease activity and disability not only in rheumatoid arthritis but also in ankylosing spondylitis, studies presented in subchapter I.3.2.3.

Chapter 4 details my oncology research. The rise in neoplastic cases prompted me to study colorectal, bone, and gynecological cancers. Multidisciplinary cancer care involves specialists from medical, surgical, and radiation oncology, pathology, radiology, palliative care, rehabilitation medicine, genomics, and bioinformatics. Colorectal cancer ranks third in incidence (6.1%) and second in mortality (9.2%). The modern approach of treatment options for patients with CRC leads to the necessity of taking into consideration their QoL after surgical and/or oncological protocol application. QoL should be included in the evaluation of such patients, along with the survival assessment, tumor recurrence, side effects and toxicity of chemoradiation, the physical and psychological effects of wearing a stoma, as well as the cost-effectiveness of procedures (subchapter I.4.2.2.1). Gold nanoparticles offer versatile nontoxic carriers for drug release, they have the potential to avoid systemic toxicity and side effects on healthy tissues when used as drug carriers, if considered the viability of normal versus malignant cells under the influence of unloaded nanoparticles (subchapter I.4.2.1).

Section II covers scientific, professional, and academic development plans.

Section III concludes the habilitation thesis with bibliographic references supporting the scientific research.

Rezumat

Teza de abilitare cu titlul *"Medicina Internă la Interfața Specialităților"* este elaborată conform recomandărilor Consiliului Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU) și metodologiei Școlii Doctorale a Universității "Grigore T. Popa" din Iași. Teza de abilitare este alcătuită din trei secțiuni după cum urmează:

În **prima secțiune**, am trecut în revistă atat parcursul meu academic, începând din 1991 ca asistent universitar la Departamentul de Semiologie Medicală din cadrul Spitalului Clinic C.F. Iași, devenind ulterior șef lucrări și din 2020 și până în prezent conferențiar, cât și cel clinic ca medic primar în medicina internă și geriatrie gerontologie. Activitatea mea științifică a început odată cu înscrierea într-un program de doctorat, pe care l-am finalizat în 2002 cu teza "Terapia Antibiotică Actuală în Tratamentul Pneumoniei Bacteriene" în domeniului Bolilor Infecțioase. Preocupările mele în acest domeniu și în interrelațiile dintre medicina internă și alte specialități au continuat în perioada postdoctorală, ceea ce a dus la publicarea a 25 de lucrări ISI, 13 ca autor principal, 4 rezumate ISI și 4 articole Proceedings, alături de 29 de articole BDI. Ca o recunoaștere a calității acestor lucrări, 3 dintre ele au fost premiate de către UEFSCDI. De asemenea, indicele Hirsch este 9, iar factorul de impact cumulativ al articolelor mele ISI ca autor principal este de 29,03.

Activitatea mea științifică este structurată în 4 direcții de cercetare după cum urmează:

Capitolul 1 prezintă rezultatele cercetărilor mele în domeniul bolilor infecțioase. Subcapitolul 1.2.1 se concentrează asupra patologiei Covid-19, cu care m-am confruntat în timpul pandemiei. In 2020, OMS a anunțat numele oficial al noului virus identificat în Wuhan, China, ca fiind "SARS-CoV-2", virus ce provoacă sindromul respirator acut. Acesta face parte din sub-familia de Coronavirusuri: 229E, NL63, OC43, HKU1, SARS-CoV, MERSCoV. Am efectuat un studiu epidemiologic retrospectiv al infecției cu SARS-CoV-2, identificând manifestările sale în Moldova. Evoluția acestor manifestări este în acord cu starea generală a pacientului până în acel moment (subcapitolul I.1.2.1.3). Hipoxia, stresul sistemic implicând factori inflamatori, tromboza, rigiditatea arterială, răspunsul inflamator și insuficienta multi-organ sunt consecințe evidente ale activității virusului pe baza datelor curente. Ca urmare studiile recente demonstrează impactul virusului asupra remodelării arteriale la pacienții cu boli vasculare preexistente și dezvoltarea potențială a unei vasculopatii COVID-19 persistente și cronice (subcapitol I.1.2.1.1). Leziunile polimorfe ale pielii și mucoaselor asociate cu SARS-CoV-2 nu sunt exclusiv datorate etiologiei virale. Variația aspectelor clinice poate apărea din cauza diferențelor patogenice, a reactivității gazdei, co-infecțiilor, vasculitei induse de virus și vasculopatiei trombotice (subcapitol I.1.2.1.2).

Continuând în cadrul domeniului bolilor infecțioase, pe lângă infecția cu SARS-CoV-2, acest capitol analizează interrelațiile dintre modificările biochimice și tratamentul antibiotic, în special în ceea ce privește microbiota cavității orale (subcapitol I.1.2.2.1).

Capitolul 2 se referă la cercetări în domeniul sindromului metabolic.

Sindromul metabolic cuprinde anomalii metabolice precum hipertensiunea, obezitatea centrală, rezistența la insulina și dislipidemia aterogenică. Afectarea hepatică poate fi asociată diabetului de tip 2, supraponderalității, obezității sau hiperlipidemiei. Este cunoscută legătura dintre diabet și boala hepatică non-alcoolică (NAFDL). NAFDL cuprinde un spectru larg de afecțiuni, inclusiv stetoza hepatică, inflamația, ciroza, fibroza și hepatocarcinomul (subcapitol I.2.2.1.2). Bolile cardiovasculare reprezintă o principala cauza de deces în rândul persoanelor diabetice. Rolul jucat de markerii inflamatori in evaluarea bolii arteriale periferice alături de caracteristicile hematologice ale pacienților coronarieni cu comorbidități metabolice, sunt prezentate in acest subcapitol. Hiperglicemia determina

modificări la nivelul florei microbiene orale si produce de asemenea un dezechilibru la nivelul profilului citokinelor inflamatorii din lichidul crevicular gingival (subcapitolul I.2.2.3).Alte patologii orale sunt prezentate in subcapitolul I.2.2.4.

Capitolul 3 detaliază preocupările mele în domeniul afecțiunilor reumatice mediate imun și implicațiile lor sistemice.

Studiul meu a evaluat relația dintre imunogenicitate, nivelurile medicamentului și eficacitatea clinică a inhibitorilor TNF în spondilita anchilozantă. Până la o treime din cazurile de spondilartropatii (SpA) nu răspund la agenții anti-TNF sau prezintă toxicitate la medicamente, ceea ce determina întreruperea tratamentului. Tulburări atopice precum rinita, dermatita atopică și astmul pot apărea la pacienții cu spondiloartropatii în tratament cu terapie biologică. Pacienții atopici pot necesita treceri mai frecvente la alte terapii biologice pentru a-și controla boala activă (subcapitolul I.3.2.1). Datele despre modificările lipoproteinelor și legătura lor cu bolile cardiovasculare și ateroscleroza în scleroza sistemică (SSc) sunt încă provocatoare. Pacienții cu SSc sunt expuși riscului de a dezvolta un profil lipidic anormal (cu colesterol HDL seric scăzut și niveluri ridicate de colesterol LDL, trigliceride și colesterol total), în special cei cu SSc cutanat difuz, cei cu boală activă și severă și anticorpi specifici (subcapitolul I.3.2.2). Tulburările reumatice inflamatorii cronice (IRD), cum ar fi artrita reumatoidă (RA), spondilita anchilozantă (AS) și alte spondiloartropatii (SpA), reprezinta o problema atat din punct de vedere economic cat si legată în principal de activitatea bolii, severitatea, dizabilitatatea produsa cat și de calitatea vieții afectate. Artrita temporomandibulara se corelează semnificativ cu activitatea bolii și produce dizabilitati nu numai la pacientii cu artrita reumatoidă, ci și la cei cu spondilita anchilozantă (studiile prezentate în subcapitolul I.3.2.3).

Capitolul 4 detaliază cercetarea mea în oncologie. Creșterea cazurilor de neoplazii m-a determinat să studiez cancerul colorectal, osos și ginecologic. Îngrijirea multidisciplinară a pacienților cu cancer implică specialiști din oncologia medicală, chirurgicală și radiologică, patologie, radiologie, îngrijiri paliative, medicină de reabilitare, genomica și bioinformatica. Cancerul colorectal ocupă locul trei în ceea ce privește incidența (6,1%) și locul doi în ceea ce privește mortalitatea (9,2%). Abordarea modernă a opțiunilor de tratament pentru pacienții cu CRC conduce la necesitatea de a lua în considerare calitatea vieții lor după aplicarea protocolului chirurgical și/sau oncologic. Calitatea vieții ar trebui inclusă în evaluarea acestor pacienți, alături de evaluarea supraviețuirii, recurența tumorii, efectele secundare și toxicitatea chimioradiației, efectele fizice și psihologice ale purtării unei stomii, precum și costul procedurilor (subcapitolul I.4.2.2.1). Nanoparticulele de aur oferă suporturi versatili și netoxici pentru eliberarea de medicamente, având potențialul de a evita toxicitatea sistemică și efectele secundare asupra țesuturilor sănătoase atunci când sunt folosite ca suporturi de medicamente, dacă este luată în considerare viabilitatea celulelor normale versus maligne sub influența nanoparticulelor descărcate (subcapitolul I.4.2.1).

Capitolul 4 descrie cercetările mele în domeniul oncologiei.

În ultimele decenii, numărul pacienților cu patologie neoplazică a crescut, astfel încât mi-am îndreptat atenția către studiul detaliat al cel puțin 2 tipuri de neoplazii, printre care amintesc cancerul colorectal si osos. Managementul patologiei oncologice necesita o abordare holistică și multidisciplinară, implicând specialiști medicali din domeniile oncologiei medicale, chirurgicale și radiologice, îngrijiri paliative, medicină de reabilitare și multe alte discipline. Pe măsură ce deciziile privind tratamentul cancerului sunt influențate din ce în ce mai mult de rezultatele testelor moleculare sofisticate, noii specialiști, cum ar fi cei din patologiile moleculare, experți în genomica și bioinformaticieni, sunt adăugați echipei de îngrijire a cancerului.

Cancerul colorectal, prezentat în subcapitolul I.4.2.2.1, este al treilea ca recunoaștere (6,1%) și al doilea ca mortalitate (9,2%). Se estimează că până în anul 2035, numărul total

de decese cauzate de cancerul rectal și de colon va crește cu 60%. Abordarea modernă a opțiunilor de tratament pentru pacienții cu CRC conduce la necesitatea de a lua în considerare calitatea vieții lor după aplicarea protocolului chirurgical și/sau oncologic. Calitatea vieții ar trebui inclusă în evaluarea acestor pacienți, alături de evaluarea supraviețuirii, recurența tumorii, efectele secundare și toxicitatea chimioradiației, efectele fizice și psihologice ale purtării unei stomii, precum și costul procedurilor . Nanoparticulele de aur pot fi dezvoltate ca transportatori versatili și netoxici pentru eliberarea medicamentelor, atât timp cât pot fi conjugate cu diferite molecule, inclusiv chimioterapice, anticorpi, peptide, liganzi și alte structuri care sunt susceptibile să promoveze o capacitate mare de penetrare a sitului tumoral, rezultând într-o acumulare predominantă a agentului bioactiv în regiunea tumorii (subcapitolul I.4.2.1).

Secțiunea II cuprinde planurile de dezvoltare științifice, profesionale și academice.

Secțiunea III încheie teza de abilitare cu referințele bibliografice care au stat la baza cercetărilor științifice.

SECTION I – Professional, academic and scientific achievements

A. Professional activity

Education, degrees and diplomas

From the beginning of my teaching career, I have been a part of the Discipline of Medical Semiology-Internal Medicine at "Grigore T. Popa" University of Medicine and Pharmacy in Iași. Over almost 30 years, I have had the opportunity to be involved in educational, scientific, research, and clinical activities, building professional and collegial relationships with members of the discipline. I believe in continuity and the idea that valuable things are accomplished when you travel the path to excellence as a team. Looking towards the future, I aspire to continue developing within the same discipline in my professional career, and in turn, to contribute through involvement, preparation, and dedication, alongside colleagues, to the achievements of the Medical Semiology Discipline at "Grigore T. Popa" University and Pharmacy Iași.

I graduated from the Faculty of Medicine at "Grigore T. Popa" UMF Iași, class of 1987. I began a 3-year internship at Sf. Spiridon Hospital in Iași, following the curriculum of that period, including clinical internships in internal medicine, pediatrics, surgery, obstetrics-gynecology, and medical care both at the hospital and in the country at the Mircesti medical office. During this time, I completed a one-year internal medicine internship under the guidance of notable figures such as Prof. Dr. Constantin Negoita and Dr. Prof. Georgeta Datcu.

Subsequently, starting in 1991, my medical profession was complemented by an educational role as I entered higher education as an assistant professor in the Discipline of Medical Semiology at the 5th Medical Clinic of Railway Iași Hospital. This was a significant benefit for me. I became a senior lecturer in 2005 following a competition at "Grigore T. Popa" University of Medicine and Pharmacy Iași, after 14 years of activity within this discipline. In 2020, after an additional 15 years, I became an associate professor. I underwent training in the field of Internal Medicine, progressing through all stages from secondary physician (Ministry of Health order no. 1711/1991), specialist physician in internal medicine since 1994 (Ministry of Health order no. 2214/1994), and achieving the title of primary physician in 1999 (Ministry of Health order no. 637/1999). Changes in the clinic's status, coupled with a growing interest in Geriatrics and Gerontology, led to the necessity of delving deeper into this field. As a result, in 2001, I passed the examination for a second specialization in Geriatrics and Gerontology (Ministry of Health order no. 866/2001 and in 2023 as primary physician in this field. I completed numerous postgraduate courses in both strictly professional domain, leading up to certifications for general echocardiography (28.09.2006, at "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Certificate of Additional Studies in General Echocardiography, series C no. 017683) and abdominal ultrasound (29.04.2002, General Ultrasound Competence Certificate, series A no. 006608, at "Grigore T. Popa" UMF Iași), as well as in the field of medical management. Notable among these are: a two-year postgraduate course in European Public Management from 2008 to 2010, diploma series H 000343, Modern Health Organization Management, "Gr. T. Popa" UMF Iași (certificate series B no. 00301 25.04.2014), Risk Management Program (certificate series PC no. 4850, February 2013), Training Program "Development of Internal Managerial Control System in Public Entities" (Certificate Series PC No. 4819, August 2011), Program for Quality Management in Healthcare EMC (July 2011), Advanced Training Course for the Occupation of Quality Auditor, Ministry of Health (M.M.P.S), Ministry of Education, Research, Youth, and Sports (M.E.C.T.S), (Graduation Certificate Series G No. 00244176, 2008-2010). Entering higher medical education and focusing efforts towards a university career naturally led me to enroll in a doctoral program in 1995. The

doctoral studies, conducted under the direct guidance of Prof. Dr. Stefan Dimitriu, one of the mentors of the Iasi School of Infectious Diseases, were completed in 2001 when I obtained the title of Doctor of Medical Sciences. My doctoral thesis titled "Current Antibiotic Therapy in the Treatment of Acute Bacterial Pneumonia" (Doctoral Diploma No. 3570, 2002) addressed topics of interest for that period.
Within the doctoral thesis, I conducted a study based on the prognostic score proposed in 1997 by the American Society of Infectious Diseases for the assessment of community-acquired pneumonia. This study involved analyzing 1112 cases of patients with community-acquired bacterial pneumonia in order to determine the current characteristics and specificities of this pathology in the Moldova region, as well as evaluating antibiotic treatment regimens. Additionally, I conducted a prospective study on the effectiveness of first-line therapy with Azithromycin (the newest antibiotic discovered at that time) in hospitalized community-acquired pneumonia compared to traditional antibiotic therapy.

B. Academic activity

As an assistant professor and later as an associate professor, I had the opportunity to work with students from both the Faculty of Dental Medicine (initially in years III and IV, and later in year II) and the Faculty of Medicine, Romanian and French sections. With them, I conducted lectures and practical work in Medical Semiology, Internal Medicine, and Geriatrics-Gerontology. I also conducted lectures and practical work in Geriatrics with students from the Faculty of Bioengineering and with students from the "General Medical Assistance" program. I supervised over 20 students in the development of their thesis papers within the Faculty of Dental Medicine at the "Grigore T. Popa" University of Medicine and Pharmacy in Iasi, for both the Romanian and French series. Additionally, I have been and continue to be a year tutor for the French series of the second year of Dental Medicine. Along the way, I aimed to improve my teaching method by using new interactive approaches that motivate and engage students to actively participate in lectures and practical work.

Furthermore, a constant concern has been the revision, renewal, and alignment of course content and stages with students, in accordance with the latest trends in the field of internal medicine, using both printed and electronic teaching materials. From 2006 to 2008, our department hosted the master's program "Clinical Foundations of Geriatrics and Gerontology," in which I served as a lecturer. I have also contributed to the training of several series of resident doctors in specialties such as geriatrics, family medicine, pulmonology, and psychiatry.

Beyond the educational process itself (both theoretical and practical), recognizing the importance of instructional support in medical education, I have contributed to the development of teaching materials by writing books as an author or co-author, which are useful for students in both the French and Romanian series. Over the years, I have guided the clinical training in internal medicine for residents from various specialties (pulmonology, family medicine, rheumatology, psychiatry), and since the year 2021, I have been appointed as an activity coordinator in the field of internal medicine. In this context, I have authored books and book chapters:

- under the editorial guidance of **Irina Esanu** - Esentialul in Semiologie si Medicina Interna pentru studentii Facultatii de Medicina Dentara (partea a-I-a Aparatul respirator), Editura Gr. T. Popa, U.M.F. Iasi 2019, ISBN 978-606-544-649-6
- sous rédaction **Irina Esanu** - L'Essentiel dans la Sémiologie et la Médicine Interne pour les étudiants de la Faculté de Médicine Dentaire (première partie-L'appareil respiratoire), Editura Gr. T. Popa, U.M.F. Iasi 2019, ISBN 978-606-544-646-5

- under the editorial guidance **Irina Esanu** - Esentialul in Semiologie si Medicina Interna pentru studentii Facultatii de Medicina Dentara (partea a-III-a Aparatul digestiv), Editura Gr. T. Popa, U.M.F. Iasi 2019, ISBN 978-606-544-654-0.
- sous rédaction **Irina Esanu** L'Essentiel dans la Sémiologie et la Médicine Interne pour les étudiants de la Faculté de Médicine Dentaire (troisième partie-L'appareil digestif), Editura Gr. T. Popa, U.M.F. Iasi 2019, ISBN 978-606-544-653-3
- Curs de Semiologie si Patologie Medicala, Editura Junimea 2004, ISBN 973-37-1010-5, Rodica Ghiuru, **Irina Esanu**. Repere in medicina interna pentru tinerii clinicieni, Note de Curs, Editura „Gr. T.Popa", UMF Iasi, 2019, ISBN 978-606-544-705-9 under the editorship of Paloma Lascarache, Chapter: BRGE, Ulcerul peptic, Sindromul de intestin iritabil, Paloma Lascarache, Cristina Gavrilescu, **Irina Mihaela Esanu**, Cringuta Paraschiv.
- Traditii si orientari in geriatria moderna, Editura „Gr. T. Popa", U.M.F Iasi, 2014, ISBN 978-606-544-205-4, coordonator Rodica Ghiuru, Author Chapter VI: Directii moderne in abordarea geriatrica a aparatului digestiv and Chapter IX: Directii moderne in abordarea geriatrica a patologiei osteoarticulare.
- Abordari interdisciplinare in Medicina Dentara - Ghiduri terapeutice, Casa Editoriala Demiurg 2013, ISBN 978-973-152-257-9 under the coordonation of Prof. Norina Consuela FORNA, Author chapter: Abordarea pacientului varstnic in vederea reabilitarii orale.
- Ghid de Urgente in Geriatrie, Editura Junimea 2007, ISBN 973-37-1235-3, under the editorship of Prof. Rodica GHIURU, Authors: Rodica Ghiuru, Cristina Maria Gavrilescu, **Irina Esanu**.
- Repere in medicina interna pentru tinerii clinicieni, Note de Curs, Editura „ Gr. T. Popa", UMF Iasi, 2019, ISBN 978-606-544-705-9 under the editorship of Paloma Lascarache, Chapter: Boala inflamatorie cronica intestinala - Cancere digestive, Paloma Lascarache, **Irina Mihaela Esanu**, Dragos Munteanu, Irina Gavril.
- Repere in medicina interna pentru tinerii clinicieni, Note de Curs, Editura „Gr. T. Popa", UMF Iasi, 2019, ISBN 978-606-544-705-9 under the editorship of Paloma Lascarache, Chapter: Hepatite cornice, Ciroze hepatice, Paloma Lascarache, **Irina Mihaela Esanu**, Cringuta Paraschiv, Diana Tatarciuc.
- Abordarea interdisciplinara a patologiei infectioase in an pandemic, VIIth Edition, Editors: Egidia Miftode, Florin Rosu, Carmen Manciuc, Editura „Grigore T. Popa", U.M.F. Iasi 2021, Chapter: Evolutia fibrozei hepatice a pacientilor cu infectie virala C post tratament antiviral oral, Oana Irina Gavril, Diana Tatarciuc, R. Gavril, M. Glod, **Irina Esanu**.
- Abordarea interdisciplinara a patologiei infectioase in an pandemic, VIIth Edition, Editors: Egidia Miftode, Florin Rosu, Carmen Manciuc, Editura „Grigore T. Popa", U.M.F. Iasi 2021, Chapter: Managementul pacientului cu afectare cardiovasculara in context pandemic, Diana Tatarciuc, **Irina Mihaela Esanu**, Cringuta Paraschiv, Paloma Lascarache, Cristina Gavrilescu, D. Munteanu, Irina Gavril.

C. Scientific achievements

Premises of my scientific activity

Scientific research activity is an integral part of the academic environment, and any university measures its value through both the educational process and research. I began engaging in scientific research even before the start of my teaching career. Thus, during my university studies, I actively participated with papers in student scientific events. Later, after commencing my teaching career, scientific activity continued and manifested over time

through the publication of chapters in specialized works, articles, or presentations at various scientific events.

The results of the research activity can be summarized as follows:

- 25 full-length ISI articles, 13 as primary author and 12 as co-author, 4 ISI abstracts, 4 ISI Proceedings
- 29 BDI articles, of which 18 as the main author
- Published articles in abstract form in national conference volumes
- Member of an internal research grant at "Grigore T. Popa" University of Medicine and Pharmacy with contract no. 30888/2014, titled "Ethical management system model in the NE region healthcare institutions - a support for the improvement of the quality of service for patients and of CONAS accreditation references," conducted from 01.01.2015 to 31.12.2016; grant director Assoc. Prof. Dr. Vladimir Poroch.
- Project "Professional Counseling for Medical Students and Integrated Practice Program in Dental and General Medicine" Project Manager Prof. Dr. Norina Forna POSDRU/160/2.1/S/139881 Sectoral Operational Program for Human Resources Development 2007-2013 Priority Axis 2. Correlating lifelong learning with the labor market, Major Field of Intervention: Transition from school to active life Function: target group.
- COST Action CA19132, European network to advance best practices & technology on medication adherence (ENABLE), 2020-2024.
- Peer-reviewer for Journal of Pharmaceutical Research International, 2020.
- Peer-reviewer for Journal of Medicine MDPI 2021, 2022, 2023.

In addition to the mentioned publications, the results of research activity have been the content of numerous oral presentations, both at international and national scientific events (13 and 18, respectively), as well as in the form of posters. I have been part of the organizing committees of the International Congresses of the Romanian Dental Association for Education and the Romanian Society of Oral Rehabilitation. I have also chaired sections of the Congress of the Balkan Stomatology Society.

I have also supervised works for the national student congresses STOMIS, where I have also chaired the preclinical section in recent years. Valuable scientific research cannot be achieved without well-organized teams where members complement each other, and each individual is crucial to the overall success of the team's work.

CHAPTER 1: RESEARCH CONTRIBUTIONS IN THE FIELD OF INFECTIOUS DISEASES

I.1.1. STATE OF THE ART

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a seafood market in Wuhan, China, has ushered in a new era, now accounting for over 790 million cases worldwide with approximately 7 million deaths (WHO, 2019).

It transformed into a pandemic, seized global attention, and was the biggest highlight of the year 2020. The first cases of the now known SARS COV-2 infection were identified in December 2019. On the 21st of December 2019, the World Health Organization (WHO) takes notice of the first suspect cases and in Romania in March 2020. The SARS-CoV-2 outbreak has jeopardized health systems and greatly affected socioeconomic parameters, transformed the clinical and increased the need for future medical research.

From the beginning of COVID-19 pandemic period in Romania I have been the head of the Internal Medical Department of the support hospital of Moldavia, which allowed me the opportunity for a new research direction in my career, related to this new challenge, this being reflected in the publication of 3 ISI listed articles.

Being a hospital support for SARS-COV-2 infection, we treated over 1000 patients diagnosed with this virus, aged between 0-90 years, complex cases with many comorbidities, which was a continuous challenge for good management of these cases.

During this critical period, the relationship between the 2 specialties, respectively internal medicine and infectious diseases was essential to implement the adequate therapy to each case.

Although the epidemiological, pathogenesis, complications, and clinical characteristics of patients with SARS-CoV-2 infection in the acute phase have been evaluated (Wong et al, 2023), few studies have characterized the epidemiology, symptomatology, and risk factors of long-term COVID-19.

Considering the data that we currently have, it is obvious that the virus can cause hypoxia, systemic stress involving inflammatory factors, thrombosis, arterial stiffness and inflammatory response and multiple system organ failure. Moreover, this raises concerns regarding the extent of arterial remodelling in patients with preexisting vascular disease and the potential development of a persistent, chronic COVID-19 vasculopathy. Long-term follow up on larger cohorts is required to investigate the reversibility of COVID-19-induced vascular changes and their associated prognostic implications.

According to statistics, associated pathology is more common in men (hypertension, diabetes, cardiovascular diseases to which are added metabolic diseases - e.g., obesity) which leads to imbalanced outcomes.

During the COVID-19 pandemic period we encountered severe and potentially life-threatening mucocutaneous dermatologic reactions mainly because of viremia, virus-host interaction-induced cytokine storms, and the consequences also probable drug reactions. The mucocutaneus lesions that were observed in some patients positive to SARS-COV-2 infection were divided in: virus-related skin lesion, skin reaction because of protective equipment and hand sanitiser, adverse drug reaction of therapies for COVID-19, and primary skin diseases which are affected by virus or its therapies.

Recently, Some COVID-19 studies reported severe and life-threatening cutaneous drug reactions such as Acute Generalized Exanthematous Pustulosis (AGEP) and Drug reaction with eosinophilia and systemic symptoms (DRESS). Widespread use of drugs such as hydroxychloroquine in treatment and prophylaxis of COVID-19, was associated with increased drug-induced skin reactions such as AGEP and erythema multiforme. (Robustelli et al, 2020; Grandolfo et al, 2020).

Lockdowns, the fear and the immune innate system particularities determined the delay of the early diagnosis and therapeutic of the cases. Although WHO has officially declared the end of the pandemic, post-COVID-19 pathology continues to be a reality and requires continuous monitoring and the holistic approach of these patients in a multidisciplinary process as it can be observed in Figure 1.1 (Sabetkish et al, 2021).

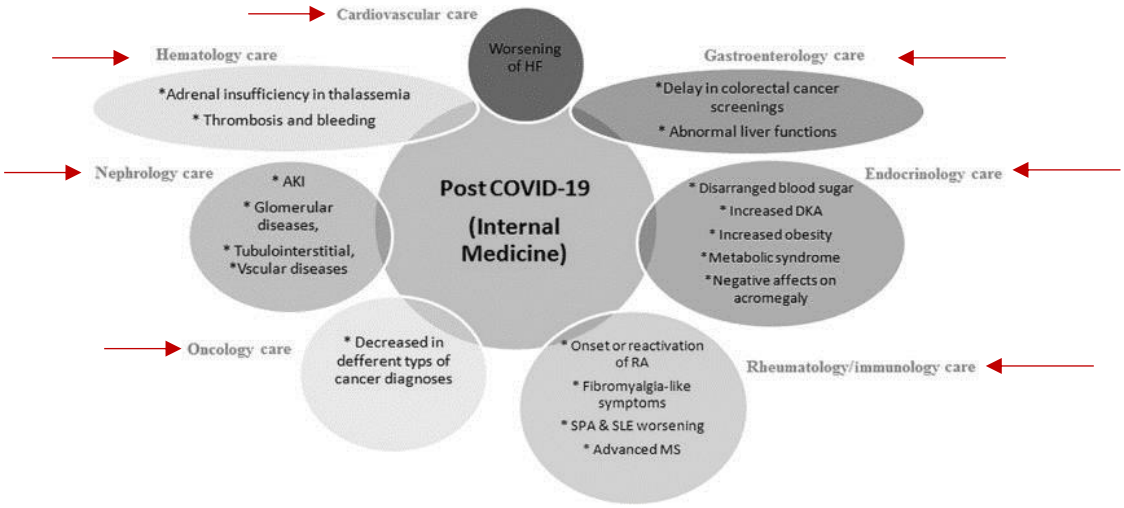

Fig. 1.1. The consequences of COVID-19 on internal medicine care after the pandemic (Adapted after Sabetkish et al, 2021)

I.1.2. SCIENTIFIC CONTRIBUTIONS

My concerns in the field of infectious diseases began in 1996, when I started my doctoral studies on "Current antibiotic therapy in the treatment of bacterial pneumonia" which were completed in 2001, but the collaboration with this discipline did not stop at this point. My interest in this specialty has been maintained over the years, materializing through the publication of ISI-listed articles as an author and respectively as a co-author.

The attraction to the field of infectious pathology reached its peak with the appearance of the COVID-19 pandemic, the moment when I started the scientific research in this pathology, with the publication of 3 ISI-listed articles.

A constant concern for my development is materialized by performing postgraduate courses, research directions that can be found in the main studies I conducted. These include International Postgraduate Course in Immunology (1996) and Postgraduate Course in Antibiotics and Antibiotic therapy (1998).

The implementation of quality and patient safety medical services was one of the concerns of my career. This was due by obtaining competencies in health management both at national and European level.

The main studies that I had in this direction of research are published in the next scientific papers:

Published articles

1. Acute and Long-Term Consequences of COVID-19 on Arterial Stiffness—A Narrative Review, Ioana Mădălina Zota, Cristian Stătescu, Radu Andy Sascău, Mihai Roca, Larisa Anghel, Alexandra Maștaleru, Maria Magdalena Leon-Constantin, Cristina Mihaela Ghiciuc, Sebastian Romica Cozma, Lucia Corina Dima-Cozma, **Irina Mihaela Esanu**, Florin Mitu, Life 2022, 12(6), 781; https://doi.org/10.3390/life12060781. IF=3,25

2. Mucocutaneous lesions associated with SARS CoV 2 infection (Review) Mihaela Paula Toader, Daniel Constantin Branisteanu, Mihai Glod, **Irina Mihaela Esanu**, Catalina Ioana Branisteanu, Maria Stefana Capsa, Andreea Dimitriu, Alin Codrut Nicolescu, Alin Constantin Pinzariu, Daciana Elena Branisteanu, Experimental and Therapeutic Medicine, February 3, 2022, https://doi.org/10.3892/etm.2022.11183. IF=2,447.
3. Retrospectiv study regarding the epidemiological aspects of Covid-19 infection in Moldavia, Romania, **Esanu I**, Dascalu CG, Gradinaru I, Apostu A, Vascu B, Ancuta C, Ciocan Pendefunda AA, Iordache C, Antohe ME., RJOR 2023, 15(2): 178-190. IF=0.7
4. Chemical and biological factors in infectious diseases the oral microbial flora, **Esanu I**, Debita M, Dorobat CM, Iliescu AA, Matei MN, Palade DO, Earar K. Revista de Chimie, 2019, 70(4): 1420-1423. IF=1.605

I.1.2.1. Clinical implications of COVID-19 pathology

I.1.2.1.1. Arterial remodelling and the potential development of COVID-19 vasculopathy

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single stranded, positive-sense, enveloped RNA virus, responsible for the current coronavirus disease 2019 (COVID-19) pandemic (Guan et al, 2020; Evans et al, 2020). Although initially described as an acute respiratory disease, COVID-19 is now considered a complex multisystemic disease, with potential longterm consequences described in up to 25% of patients (long COVID-19, post-COVID-19 syndrome) (Barrantes et al, 2021).

Arterial stiffening, although naturally associated with aging, can be accelerated by associated respiratory, metabolic and cardiovascular comorbidities (Figure 1.2) (Zota et al, 2021). The chronic increase in afterload precipitates left ventricular remodelling and the development of heart failure (Çiftel et al, 2022). As such, arterial stiffness and the modern concept of early vascular aging have been introduced as major determinants of vascular health. Arterial stiffness can be assessed in both muscular and elastic vessels, cross-sectionally or longitudinally, but in measure of parietal stiffness (Townsend et al, 2016). Systolic pressure augmentation, also known as the augmentation index, compares brachial artery pressure with central aortic blood pressure; although dependent on arterial stiffness, systolic pressure augmentation is also influenced by several other factors, especially heart rate (Wilkinson et al, 2000). Although arterial stiffness is a powerful prognostic marker, it is not routinely performed in clinical practice, partially due to the variety of methods and devices that claim to assess arterial stiffness and vascular age (Segers et al, 2020). PWV is commonly calculated as the ratio between the distance and the pulse wave travel time for the pulse wave between the proximal and distal measurement sites. A direct, invasive aortic PWV measurement via pressure catheter recordings, offering a comprehensive anatomical delineation and correct estimation of pulse wave transit time, is rarely used in clinical practice due to cost and complexity. However, aside from the high cost and low availability, a high temporal resolution is required to accurately compute through-plane MRI signaling. Due to technical difficulties in assessing aortic PWV, its surrogate, carotid-femoral PWV (cfPWV) has become the gold standard for evaluation of arterial stiffness as recommended by current guidelines (Townsend et al, 2016). Other surrogates are brachial-ankle PWV (baPWV) and estimated PWV (ePWV) (equation derived from the Reference Values for Arterial Stiffness Collaboration) (Greve et al, 2016; Reference Values for Arterial Stiffness' Collaboration, 2010). The current guidelines for the management of arterial hypertension

(HTN) (Williams et al, 2018) list a carotid-femoral PWV of >10 m/s as a feature of asymptomatic HTN-mediated organ damage, but with a weak class of recommendation.

Hypertension and age are major determinants of vascular stiffness and also independent risk factors for COVID-19 mortality (Rodilla et al, 2021). Moreover, diabetes, obesity, smoking and dyslipidemia are known risk factors for endothelial dysfunction and for worse COVID-19 outcomes, and low-grade inflammatory components have regularly been labelled in the physiopathology of early vascular aging (Epidemiology Working Group for NCIP Epidemic Response, 2020; Richardson et al, 2020).

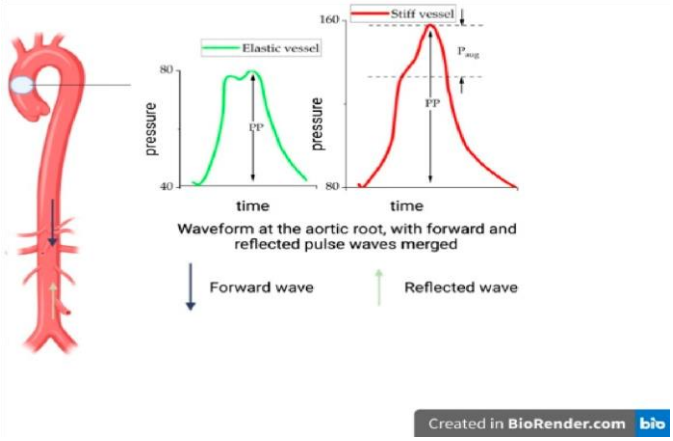

Fig. 1.2. The concept of arterial stiffness. In stiff vessels, the reflection of the pulse wave occurs prematurely, during systole, leading to an early merger of the forward and reflected pulse waves, isolated systolic hypertension, adverse afterload pattern, reduced coronary perfusion and organ damage in low resistance vascular beds.

Therefore, a noninvasive arterial stiffness assessment could help optimize risk stratification in COVID-19 patients, which could favor a more aggressive treatment approach in "high-risk" patients. The scope of this review is to summarize available data on the acute and long-term consequences of COVID-19 on arterial stiffness and other parameters of vascular function.

Materials and Methods

The population targeted in the following review consists of data from the literature regarding patients of all ages with current or previous SARS-CoV-2 infection, isolated or compared to a control group of patients without a previous COVID-19 diagnosis. The primary intervention was an arterial stiffness assessment, either isolated or accompanied by the additional evaluation of endothelial and cardiac dysfunction.

Electronic Search Strategy

We conducted a comprehensive literature review of the articles currently available in the EMBASE, MEDLINE and PubMed databases, according to PRISMA guidelines. We used the following keywords: "COVID-19", "SARS-CoV-2", "arterial stiffness", "PWV" and "pulse wave velocity". This review was carried out according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) checklist (Liberati et al, 2009). We applied the following selection criteria: study type - retrospective, cross-sectional or prospective analysis, case reports and case series; language - english; types of participants - patients of all ages with current or previous SARS-CoV-2 infection; follow-up duration - without restrictions; outcome - COVID-19 severity and mortality; reviews, studies available only as abstracts (including conference abstracts) and dissertations were excluded from this analysis.

Arterial Stiffness Assessment

We selected studies evaluating arterial stiffness as well as other parameters of vascular function: Primary indicator - arterial stiffness assessment (pulse wave velocity: PWV;

augmentation index: Aix; cardio-ankle vascular index: CAVI; arterial stiffness index: ASI; Young's modulus of elasticity; pulse pressure: PP) (Segers et al, 2020; Lauret et al, 2006 Vappou et al, 2010, Miyoshi et al, 2016) and Secondary indicators - intima media thickness: IMT, endothelial dysfunction assessment (flow-mediated dilation: FMD; nitroglycerin mediated dilation: NMD; perfused boundary region) (Theodorakopoulou et al, 2020).

Results

We identified a total of 15 literature reports compatible with the beforementioned selection criteria: 4 cross-sectional studies, 3 retrospective studies, 6 prospective studies and 2 case reports.

Early Impact of COVID-19 on Vascular Stiffness

An increasing number of clinical studies have assessed the impact of COVID-19 on arterial stiffness. As a first contribution to the subject, *Ratchford et al.* (Ratchford et al, 2021) observed that cfPWV (assessed by the ultrasound foot-to-foot electrocardiogram-gated method) (Laurent et al, 2006) was 0.75 m/s higher in young adults with prior COVID-19 compared to healthy controls. An increase in cfPWV seemed gender-independent; however, small sample sizes limit the value of this conclusion. The authors additionally signal a discrepancy between impaired brachial FMD and a lack of change in brachial reactive hyperemia, which could suggest that microvascular dysfunction in COVID-19 is primarily mediated by diminished NO bioavailability (Ratchford et al, 2021).

The analysis of UK Biobank data, realized by *Raisi-Estabragh et al.* (Raisi-Estabragh et al, 2019), on 70 COVID-19 patients and on 240 controls found the arterial stiffness index (ASI) (PulseTrace PCA2, CareFusion, San Diego, CA, USA) to be 0.6 m/s higher in COVID-19 fatalities (n = 8) compared to that of survivors (9.7 ± 2.7 m/s versus 9.1 ± 2.7 m/s, $p < 0.05$). However, the research did not find a significant association between ASI and COVID-19 status, mortality or critical care admission rates after age and sex adjustments (Raisi-Estabragh et al, 2019). Despite these results, three other studies (Rodilla et al, 2021; Stamatelopoulos et al, 2021; Schnaubelt et al, 2021) have highlighted arterial stiffness as a short-term prognostic marker in COVID-19.

Schnaubelt et al. (Schnaubelt et al, 2021) compared PWV (BOSO ABI Systems 100 PWV, Bosch & Sohn GmbH, Jungingen, Germany) in 22 acutely ill COVD-19 patients and 22 acutely ill nonCOVID-19 controls. BaPWV (brachial-ankle PWV) and cfPWV (carotido-femoral PWV) were independently associated with COVID-19 status in multiple regression models and were significantly higher in positive COVID-19 subjects compared to control groups (22 ageand sex-matched controls and 102 acutely ill COVID-19-negative controls). COVID-19 fatalities had greater baPWV and cfPWV ($p = 0.004$ and $p = 0.05$, respectively), and PWV was correlated with the length of hospital stay among the COVID-19 survivors, distinguishing arterial stiffness as an independent risk factor for clinical deterioration (Schnaubelt et al, 2021). The results of two retrospective cohorts (Rodilla et al, 2021; Stamatelopoulos et al, 2021) additionally support this hypothesis.

Rodilla et al. (Rodilla et al, 2021) analyzed 12,170 hospitalized COVID-19 patients and found that a pulse pressure ≥60 mmHg was an independent predictor for all-cause in-hospital mortality (adjusted OR 1.23, $p = 0.0001$). In another study, estimated PWV (ePWV) was higher among individuals hospitalized with COVID-19 compared to matched controls and offered prognostic information for 28-day mortality (Stamatelopoulos et al, 2021). In a recent protocol, the prognostic value of ePWV was additional to current validated clinical predictors (4C Mortality score) (Knight et al, 2020).

Szeghy et al. (Szeghy et al, 2021) reported that the carotid stiffness and aortic augmentation index (SphygmoCor—AtCor Medical, Sydney, Australia) are higher among young adults with recent COVID-19 (n = 15) than among healthy controls (n = 15). COVID-19 patients also had greater Young's modulus of elasticity, indicating an increased risk of

developing arterial hypertension over the following 3 years. Despite previous reports of acute increases in carotid intima thickness during hyperinflammatory states, cIMT had similar values in cases and controls, possibly due to the mild COVID-19 clinical presentation among the analyzed patients. Indeed, a prospective case-control analysis showed that the left and right CAVI (VaSera VS-1000-Fukuda-Denshi Company Ltd., Tokyo, Japan) were significantly higher in moderate–severe COVID-19 versus mild COVID-19 (Aydın et al, 2022). The left and right CAVI were more impaired among COVID-19 patients, and cut-off values of >8.5 and >8.75, respectively, could predict disease severity (Aydın et al, 2022). Vascular function can be influenced not only by COVID-19 severity in the acute phase, but also by the persistence of symptoms.

Supporting this hypothesis, *Nandadeva et al.* (Nandadeva et al, 2021) showed that peripheral micro- and macrovascular function (reactive hyperemia and brachial FMD) were impaired only among young adults with lingering COVID-19 symptoms (4 weeks after initial diagnosis). In contrast, cerebral vasomotor reactivity and central arterial stiffness (PWV, SphygmoCor, Atcor Medical, Sydney, Australia) were similar in all analyzed subgroups (symptomatic: n = 8, asymptomatic: n = 8 and controls: n = 12).Available evidence shows that COVID-19 causes significant short-term alterations to vascular physiology even in otherwise healthy young adults. Judd et al. [30] reported substantial differences regarding PWV (Mobil-O-Graph, I.E.M., Aachen, Germany) and Aix, as well as carotid, axillary and superficial femoral IMT in patients 6 months after SARSCoV-2 infection versus controls. Although vascular reactivity (FMD and NMD) did not significantly vary among the three analyzed subgroups, the authors additionally document persistent capillary changes (higher rates of capillary ramifications, capillary loss, bushy capillaries and capillary elongations) and disturbed arginine, kynurenine and homocysteine metabolism only among post-COVID-19 patients (Jud et al, 2021). The prospective nonrandomized observational COSEVAST study (Kumar et al, 2021) enrolled 64 patients without known comorbidities requiring hospitalization for COVID-19. Aix and cfPWV estimated from the brachial-ankle PWV (Periscope, Genesis Medical Systems, Hyderabad, India) gradually increased with COVID-19 severity (mild, moderate and severe, according to the National Institute of Health's criteria), even after adjustments for potential confounding factors (weight, gender, mean arterial pressure and heart rate). The authors noted that the vascular damage in severe COVID-19 cases was comparable to that observed in long standing chronic diseases (coronary atherosclerosis, diabetes and renal failure) (Kumar et al, 2021).

Long Term Impact of COVID-19 on Vascular Stiffness

COVID-19 patients present persistent arterial stiffness and endothelial dysfunction at least 4 months after initial infection, as shown by Lambadiari et al. (Lambadiari et al, 2021). This interesting study showed that both cfPWV (Complior—Alam Medical, Vincennes, France) and brachial FMD were more impaired among patients with associated HTN and among patients with previous COVID-19 compared to healthy controls. These results suggest a long-term impact of COVID-19 on both arterial stiffness (vascular) and endothelial function. Coronary flow reserve (CFR), an early marker of endothelial dysfunction with prognostic implication, was lower among patients with associated HTN and COVID-19 patients compared to controls ($p = 0.01$ and $p = 0.03$, respectively). Moreover, the perfused boundary region of sublingual arterial microvessels with a diameter of 5–25 μm (PBR5-25), a marker of endothelial glycocalyx impairment, was higher in both COVID-19 and hypertensives compared to controls ($p = 0.001$ and $p = 0.001$). COVID-19 and hypertension seem to inflict a similar degree of vascular damage.

The same study reported a significant association between persistent cardiovascular symptoms and poorer cfPWV, FMD, right and left ventricular strain values and MDA

(oxidative stress). However, cfPWV did not vary with COVID-19 severity, suggesting that vascular dysfunction persists independently of initial disease severity, although this hypothesis should be confirmed in larger cohorts.

At the 12-month follow-up (Ikonomidis et al, 2022), COVID-19 patients presented persistent arterial stiffness and endothelial dysfunction: cfPWV and central SBP remained significantly higher in COVID19 patients compared to controls ($p = 0.057$ and $p = 0.003$, respectively), and PBR5-25 increased compared to the initial evaluation at 4 months. The authors reported significant improvements in oxidative stress (MDA levels), CFR and myocardial work parameters (myocardial wasted work and efficiency), as well as a borderline improvement in left ventricular strain, which, however, remained impaired compared to the controls (Ikonomidis et al, 2022). Right heart function (right ventricular strain, tricuspid annular plane excursion) completely recovered at 12 months, possibly due to resolution of pulmonary lesions (Ikonomidis et al, 2022).

The ongoing CARTESIAN (Bruno et al, 2020) study is a large longitudinal multicenter project that analyzes cfPWV, central hemodynamics as well as biomarkers of accelerated vascular aging, 6 and 12 months after confirmed SARS-CoV-2 infection.

A pre-planned study extension aims to evaluate 10-year mortality causes, hospitalization rates and overall health status in COVID-19-positive patients.

Discussions

(Cardio)vascular Involvement in COVID-19

COVID-19 causes a plethora of cardiovascular manifestations (Grasselli et al, 2020), ranging from arrythmias, asymptomatic myocardial injury, overt congestive heart failure and thromboembolic events (Figure 1.3) (Guzik et al, 2020), attributable to the virus' direct cytotoxic effect or to the systemic inflammatory cytokine storm (Evans et al, 2020). Emerging evidence suggests that the endothelium is a primary target for SARS-CoV-2 (Varga et al, 2020; Klok et al, 2020).

Vascular endothelial cells express ACE2-R (angiotensin-converting enzyme 2 cellular receptor) and TMPRSS2 (transmembrane serine protease 2), which synergistically mediate SARS-CoV-2 entry in host cells (essential for SARS-CoV-2 pathogenicity) (Mollica et al, 2020; Hoffmann et al, 2020). The infected endothelial cells demonstrate an increased production of proinflammatory cytokines and prothrombotic factors.

The multiorgan failure observed in some COVID-19 cases is partly caused by vasculitis in multiple vascular areas (Varga et al, 2020; He et al, 2006).

Although two recently published papers argued that human endothelial cells do not express ACE2-R, Ma et al. demonstrated an ACE2-R-independent direct inflammatory activation of the endothelium, stating that other surface receptors (neuropilin-1, scavenger receptor B type 1 and CD147) could assist the direct cellular entry of SARS-CoV-2 (Ma et al, 2022).

From a macrovascular perspective, evidence suggests that COVID-19 causes early vascular aging (Çiftel et al, 2022). COVID-19-induced mitochondrial dysfunction increased local production of reactive oxygen species (ROS) and subsequent oxidative telomere shortening have been proposed as other potential causes of cellular senescence and vascular stiffening (Chang et al, 2021).

Imbalance of the host redox status favors ED and chronic subintimal inflammation, which causes accelerated fragmentation of parietal elastin fibers and their replacement with rigid, fibrotic tissue (Varga et al, 2020; Chang et al, 2021). As COVID-19-induced pulmonary fibrosis is only partially reversible (Ambardar et al, 2021), it has been postulated that arterial stiffening could be a long-term cardiovascular sequela for most patients, irrespective of COVID-19 severity (Varga et al, 2020).

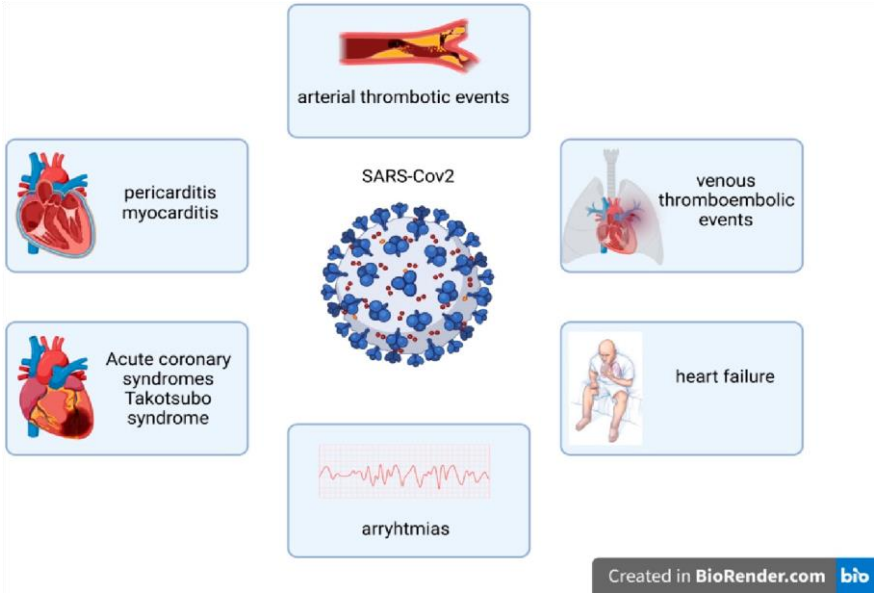

Fig. 1.3. Cardiovascular complications of SARS-CoV-2

The European Society of Cardiology endorses that close follow-up and further research is needed to address the potential therapeutic and prognostic implications of COVID19-induced endotheliitis, recommending an arterial stiffness assessment as a marker of COVID-19 outcome and treatment monitoring (Evans et al, 2020). Saeed et al. have recently argued that COVID-19 and arterial stiffness have a bidirectional cause–effect association (Saeed et al, 2021).

COVID-19-induced vascular remodeling is favored by dysregulation of the neuro-hormonal systems, endothelial dysfunction, renal damage, altered lipid and glucose metabolisms and decompensated hypertension (Saeed et al, 2021). Pre-existing atherosclerosis is an independent risk factor for COVID-19 severity, and statins have been postulated to improve COVID-19 outcomes though their pleiotropic anti-inflammatory properties and a potential inhibition of SARS-CoV-2 proteases (De Spiegeleer et al, 2020; Daniels et al, 2020). However, other studies failed to prove a substantial benefit with statin use (Peymani et al, 2021; Ayeh et al, 2021), raising some safety concerns, especially since statin therapy upregulates ACE2 receptor expression (Tikoo et al, 2015), which could enhance SARS-CoV-2 entry into respiratory epithelial cells (Hoffmann et al, 2020).

Consequences of COVID-19 on Arterial Stiffness

The available evidence shows that COVID-19 causes significant alterations to vascular physiology, even in otherwise healthy young adults. Cardiovascular involvement is central in COVID-19, and preexisting cardiovascular disease is associated with worse clinical outcomes. Systemic hyperinflammation reduces NO (nitric oxide) bioavailability, which increases vascular stiffness even in otherwise healthy individuals (Vlachopoulos et al, 2005). The endothelium is essential in vascular tone regulation and vascular remodeling, as well as in platelet aggregation and inflammation (Çiftel et al, 2022). The two major causes of endothelial dysfunction comprise direct mechanical injury and inflammation (including autoantibodies and bacterial infection) (Leask et al, 2003), which can simultaneously occur in COVID-19. The importance of endothelial dysfunction in COVID-19 pathogenesis is supported by the fact that diabetes, obesity, smoking, and dyslipidemia are known risk factors for ED and for worse COVID-19 outcomes (Epidemiology Working Group for NCIP Epidemic Response, 2020; Richardson et al, 2020). As COVID-19 is associated with ED, altered lipid and glucose metabolism, as well as decompensated HTN, it induces early, accelerated atherosclerosis (Saeed et al, 2021). Furthermore, aging and a proinflammatory

phenotype induced via angiotensin II signaling underlie the vicious circle of hypertension and arterial stiffening, which increases left ventricular afterload and impairs coronary perfusion (Wang et al, 2014; Saavedra et al, 2020).

The available reports show that some arterial stiffness parameters are correlated with length of COVID-19 hospital stay and are independent predictors for in-hospital and short-term COVID-19 mortality (Rodilla et al, 2021). *Lambadiari et al.* (Lambadiari et al, 2021) and their 12-month followup study (Ikonomidis et al, 2022) show that COVID-19 patients present persistent arterial stiffness and endothelial dysfunction.

Due to the limited number of patients enrolled in current, published reports, the gender-specific cardiovascular effects of SARS-CoV-2, as well as the impact of COVID-19 severity on arterial stiffness, need further studies. Associated comorbidities, especially chronic kidney disease, coronary and peripheral artery disease, cause significant, accelerated vascular remodeling and a prothrombotic state, increasing the risk of a COVID19-negative outcome (Smolderen et al, 2021; Jdiaa et al, 2022). In our opinion, the prognostic significance of SARS-CoV-2 associated arterial stiffening in patients with preexisting alterations of vascular function should be analyzed in future dedicated cohorts. Vascular function impairment could depend not only on the severity of COVID-19 in its acute phase, but also on the persistence of symptoms. As such, clinicians should consider at least a basic screening of arterial stiffness in patients suffering from "long COVID-19".

After the emergence of new SARS-CoV-2 strains, clinicians have noted a shift in COVID-19's clinical presentation, with Omicron generating more upper respiratory tract symptoms and, apparently, fewer thrombotic complications (del Rio et al, 2022). In this context, the impact of COVID-19 on arterial stiffness could significantly vary according to the causal SARS-CoV-2 variant. This poses further difficulties in interpreting the current literature findings. The ongoing CARTESIAN study should clarify these aspects considering the impressive number of enrolled patients.

Whether COVID-19-induced vasculopathy resolves after a couple of months or tends to evolve into a chronic vasculopathy with severe implications on cardiovascular morbimortality, requires long-term follow-up on large cohorts of patients, especially since "long COVID-19" is reported in up to 25% of cases (Logue et al, 2021).

Conclusions

COVID-19 causes early vascular aging and arterial stiffness. Future research should focus on screening, prevention and treatment of COVID-19 vasculopathy. A better understanding of COVID-19's vascular involvement and its prognostic significance will help characterize COVID-19 in its entirety, which is an essential step in its successful management. Further studies are needed in order to investigate the reversibility of COVID-19-induced vascular changes and their impact on long-term prognosis, while keeping in mind that new, emerging variants could have completely different effects on vascular physiology.

I.1.2.1.2. Vasculopathies with dermatological expression in patients with SARS-COV-2 infection

Introduction

Despite the research data currently provided, cutaneous and mucosal manifestations of SARS-CoV-2 infection remain poorly known, particularly their prevalence, their morpho-logical characteristics, the pathogenic substrate, as well as their diagnostic and prognostic significance. The ongoing pandemic, as well as the emergence of new viral strains, possibly more contagious and responsible for more severe disease (Docea et al, 2020), even with the development of several promising vaccines, that, however, have lower efficacy in a large group of individuals suffering from metabolic disorders, autoimmune diseases or with iatrogenic immune suppression (Calina et al, 2020), make it imperative to

understand the complex clinical manifestations of COVID-19, including the signs and symptoms on the skin and mucous membranes. Case reports refer to a variety of morphological aspects that are either virus-induced or associated with antiviral therapy or secondary to the circumstances of the pandemic such as stress (herpes simplex, herpes zoster and alopecia areata) and environmental factors related to the use of antiseptics and disinfectants (contact dermatitis or urticaria) (Potekaev et al, 2020; Gisondi et al, 2020; Recalcati et al, 2020; Ahouach et al, 2020, Iordache et al, 2019; Martinez-Lopez et al, 2020).

According to a French study (conducted by Raymond-Poincaré University Hospital, Garches, France), which involved ~40 patients confirmed positive for COVID-19, the most common mucocutaneous manifestations were: macular exanthema (32 patients; trunk and head and neck were the areas preferentially involved, hand and feet were spared), face edema (13 patients), oral lichenoid reaction (13 patients), enanthema (11 patients), macroglossia (10 patients), cheilitis (5 patients), livedo reticularis (5 patients), urticarial rashes (3 patients), maculopapular exanthema (3 patients), purpura (2 patients), atopic dermatitis (1 patient), herpes (1 patient). All the patients presented extremely itchy lesions (Mascitti et al, 2020).

The positive diagnosis of skin and mucosal lesions in patients with COVID-19 is difficult and primarily requires the exclusion of drug-induced dermatoses (Le Cleache et al, 2020; Brănișteanu et al, 2018) and of other eruptions with similar clinical expression, particularly other viral infections. Cutaneous lesions in patients with SARS-CoV-2 infections are extremely variable in morphological patterns and their importance as a marker for the viral infection and for disease prognosis is still debated (Ahouach et al, 2020; Kaya et al, 2020; Genovese et al, 2021; Darlenski et al, 2020; Suchonwanit et al, 2020). Mucosal lesions are markedly less studied, but there are reports of oral mucous membrane changes and ocular conjunctival or corneal lesions in patients diagnosed with COVID-19, either as solitary findings or in association with cutaneous manifestations, with unclear pathogenic mechanisms, to date (Mascitti et al, 2020; Jimenez-Cauhe et al, 2020; Rochefort et al, 2021).

A classification of the cutaneous lesions associated with SARS-CoV-2 infection based on the clinical aspect, pathogenic hypotheses, histopathological findings, associated disease severity, and prognostic importance, as well as a description of the most commonly encountered oral and ocular mucosal lesions during COVID-19 disease were reported in the present review.

Material and method

A literature search was conducted, using electronic databases Key Elsevier, Medscape, PubMed, Google Scholar, for the term ‘COVID-19’ in combination with ‘skin’, ‘cutaneous manifestations’, ‘mucosal manifestations’, ‘rash’, ‘exanthem’, ‘enanthem’, ‘urticarial’, ‘chilblain’, ‘livedo’, ‘ocular mucosa’, and ‘purpura’ to collect reports of skin and mucosal manifestations described in patients with COVID-19. Case reports, case series, and literature review-type articles were included in our research. A brief review was created, based on 63 articles identified in the literature.

Results

Oral mucosal lesions in patients with SARS-CoV-2 infection. Changes in oral mucous membranes in the context of SARS-CoV-2 disease have also been reported (Jimenez-Cauhe et al, 2020; Rochefort et al, 2021). Petechial, macular and maculo-petechial enanthems were described in patients with COVID-19 disease, accompanied by a papulovesicular rash, periflexural purpura, and erythema-multiforme-like rash. These mucosal lesions occurring concurrently with a skin rash are indicative of a viral etiology, rendering the examination of the oral mucosa an important step in differentiating between drug-induced exanthems and viral-induced skin rashes in the context of the SARS-CoV-2 pandemic (Jimenez-Cauhe et al, 2020). Lingual pain was described in patients with COVID-19, possibly due to the higher

expression of ACE2 receptor in the epithelial cells of the tongue (Rochefort et al, 2021). Oral ulcers, similar to recurrent herpes simplex or recurrent aphthous stomatitis have been reported by several authors. Pathogenic hypotheses focus on vascular and arterial thrombosis in small and medium-sized vessels (Jimenez-Cauhe et al, 2020; Rochefort et al, 2021). Lichen-planus-like lesions have been reported in patients that had been diagnosed with COVID-19 in the previous 12 months (Jimenez-Cauhe et al, 2020; Rochefort et al, 2021). In a Spanish study, 45.7% of 666 patients presented mucocutaneous lesions. On the oral mucosa, transient lingual papillitis was identified in 11.5% of cases, recurrent aphthous stomatitis in 6.9% of cases, glossitis with lateral indentations in 6.6% of cases, and depapilating glossitis in 3.9% of cases (Jimenez-Cauhe et al, 2020). The pathogenic mechanism for these manifestations is not yet fully understood.

Discussions

Although millions of cases have been registered, no pathognomonic dermatological signs and symptoms for the disease have been identified yet. The polymorphic skin and mucosal lesions associated with SARS-CoV-2 infection are not an argument for the viral etiology, as usually, a certain virus is responsible for a single type of dermatologic manifestation. However, the increased incidence of the afore mentioned clinical patterns of dermatologic conditions during the pandemic, suggests the association with the SARS-CoV-2 virus.

The diverse clinical aspects may be explained by pathogenic differences between distinct strains of the virus, differences related to the host reactivity, and the possibility of co-infections. In contrast, skin and mucosal manifestations during COVID-19 may not only be related to the virus itself, but also to the viral-induced vasculitis and thrombotic vasculopathy, or they may be due to adverse reactions to the prescribed drugs (Ahouach et al, 2020; iordache et al, 2019; Martinez-Lopez et al, 2020; Su et al, 2020). The most common side effects associated with several of the often-prescribed drugs for COVID-19 infection (antimalarials) were maculopapular exanthematous reactions, urticaria, and psoriasis exacerbation. Oral antiretroviral combination lopinavir/ritonavir may be responsible for Stevens-Johnson syndrome (Martinez-Lopez et al, 2020).

Temporal association between urticarial lesions and maculopapular eruptions with SARS-CoV-2 infection, when they appear concurrently as the systemic symptoms may be indicative of a viral aetiology, rather than a drug-induced one (Martinez-Lopez et al, 2020). It is currently considered that two types of skin manifestations may be characteristic of the COVID-19 disease chilblain-like lesions and papulovesicular lesions. Therefore RT-PCR for SARS-CoV-2 (if the onset is less than 4 weeks previous) or serological testing (IgM, IgG) for a potential SARS-CoV-2 infection should be added to the investigation protocol in patients without known risk factors who develop pernio-like lesions or in patients with papulovesicular rashes. Cases of COVID-19 with a clinical picture consisting of an infectious rash alone have been reported, making it imperative to investigate a febrile rash for the novel coronavirus as a possible cause (Gianotti et al, 2020; Su et al, 2020).

Conclusions

The description of the mucocutaneous manifestations associated with COVID-19 reviewed in this article may be helpful in the early recognition of cutaneous signs that are associated with severe complications (such as livedoid, necrotic or maculopapular lesions) and to establish prompt management essential in improving patient's prognosis. Patients with autoimmune and chronic inflammatory disorders, such as psoriasis, atopic dermatitis, lupus, scleroderma, and hidradenitis suppurativa may require special care and adjustment of their immune-suppressive therapy protocol in order to maximize the chances for an effective response to anti-Covid-19 vaccines.

I.1.2.1.3. Clinical and paraclinical particularities of COVID-19 pathology

Introduction

Population health status is a complex social and biological phenomenon that expresses the level and characteristics of health of members of a community as a whole. However, precisely defining and assessing this phenomenon is difficult because of its complexity and the various factors that affect it.

The level of health of the population at both national and global level has been seriously disrupted since 2020 by the outbreak of the coronavirus pandemic. The epidemiological aspects of Covid-19 infection are extremely important, on the general background found in all forms of manifestation there were elements of particularity in relation to the type of strain involved and especially the mode of manifestation of the clinical picture from individual to individual, from country to country (Daga et al, 2019; CDCP, 2004; WHO SARS, 2019; WHO MERSCoV, 2019; WHO COVID-19, 2020, Xiao et al, 2020, Lovelace et al, 2020; CDC COVID-19, 2020; Forna et al, 2018; Ancuța et al, 2021, Martu et al, 2019).

On February 11, 2020, WHO announced that the official name of the new virus first identified in Wuhan, China, is SARS-CoV-2. This virus is acute respiratory syndrome. It is part of the sub-family of viruses called Coronaviruses (CoV): 229E, NL63, OC43, HKU1, SARS-CoV, MERSCoV.

Coronaviruses are known to be widespread in nature, causing respiratory tract diseases and gastroenteritis in humans and animals (birds, pigs, cattle, horses, rodents, bats, cats, dogs), some very severe and others mild or even without clinical symptoms. They are the second viral group, after rhinoviruses, responsible for inducing rhinopharyngitis, the common cold in humans (Martu et al, 2019; Picard et al, 2020; AlTakarli et al, 2020; Goh et al, 2020; Zhang et al, 2020; Guan et al, 2020).

The literature shows that SARS-CoV-2 is not the first virus in its sub-family to cause numerous deaths. From 2012 to November 2019, 2,494 cases of MERS-CoV infection and 858 deaths have been reported to the World Health Organization (WHO). A virus such as SARS-CoV-2 is considered dangerous because of its virulence, speed of transmission, in a very short time. From December 2019 to March 2020, in just 4 months, 114,243 infections and 4,023 deaths have been confirmed in 25 countries (Guan et al, 2020; Shen et al, 2020; Moher et al, 2009; McGowan et al, 2015; Deeks et al, 2011; Higgins et al, 2011). The virus is transmitted in two main ways: through contact with an infected person, contact defined as a distance of about 1.8 meters, through droplets from the respiratory tract removed when a person sneezes or coughs; by touching contaminated surfaces, followed by touching the eyes, nose or mouth.

Not only elderly people are affected by COVID-19, but also young people and children. However, it appears that age category influences the severity of illness and the increased risk of death (Higgins et al, 2011; Dey et al, 2020; Chan Li et al, 2020; Holshue et al, 2020; Wu et al, 2020; Kim et al, 2020; Lin et al, 2020; Backer et al, 2020; Kim et al, 2020; Jiang et al, 2020; Thompson et al, 2020).

The aim of this study is to carry out an epidemiological retrospective of Sars-CoV-2 infection with individualization of the peculiarities of manifestation on the territory of Moldova, the evolutionary peculiarities being in full agreement with the general status of the patient up to that moment.

Material and method

779 patients with COVID-19, hospitalized between September 2020 and April 2021, 386 men (49.6%) and 393 women (50.4%), aged between 7 and 93 years, were investigated; the mean age of the patients was 52.26 ± 15.844 years, with no significant differences

between men (52.26 ± 15.830 years) and women (52.26 ± 15.878 years). More than half of the patients (59.6%) are aged between 30-59 years; 8.3% are young people under 30 years old, and about a third (32.1%) are elderly, over 60 years old– without statistically significant differences between the sexes (Pearson Chi - squared = 3.102, p = 0.875).

Results

We carried out a comparative study of the symptoms recorded in patients and comorbidities, correlated with pulmonary complications and the evolution, characterized by febrile, subfebrile and afebrile states, in order to identify and quantify the risk factors that facilitate the onset of complications. Among the 779 investigated patients, 205 (26.3%) were asymptomatic, the rest presenting between 1-9 monitored symptoms (Figure 1.4); the most frequently recorded symptoms were cough (40.2% of cases), followed by headache (21.6%), physical asthenia (21.3%) and fever (17.8%); 11.2% of patients had 1 symptom, 17.3% had 2 symptoms, 25.9% had 3 symptoms, and 19.2% had 4 or more symptoms.

We analyzed the patients' symptoms comparatively by gender and age range, using the age of 60 years as a cutoff to identify the elderly (Table 1.I). In men, cases of fever are significantly more frequent than in women (21.8% versus 14.0%) and slightly more frequent are cases of chest pain (12.7% versus 10.2%), dyspnea (11.9 % vs. 8.7%), rhinorrhea (5.4% vs. 3.3%), and sweating (5.2% vs. 3.8%); on the other hand, in women, cases of physical asthenia (25.7% versus 16.8%), anosmia (13.7% versus 7.5%), odynophagia (8.4% versus 3, 9%) and diarrhea (3.8% vs. 1.0%); other slightly more common symptoms were headache (22.4% vs. 20.7%) and myalgias (14.2% vs. 12.7%). Also, the percentage of asymptomatic was slightly higher in women (27.0%) compared to men (25.6%). In patients under 60 years of age, cases of headache (25.3% versus 13.6%), anosmia (13.2% versus 5.2%), odynophagia (7.6% versus 3.2%) and rhinorrhea (6.0% versus 0.8%), and in patients over 60 years physical asthenia (30.8% versus 16.8%), dizziness (7.6% vs. 3.6%), loss of appetite (10.0% vs. 5.1%), and pain other than chest pain (17.2% vs. 7.4%), along with other manifestations (8.8% versus 5.1%). The percentage of asymptomatic patients is significantly higher among those under 60 (28.7%) compared to those over 60 (21.2%).

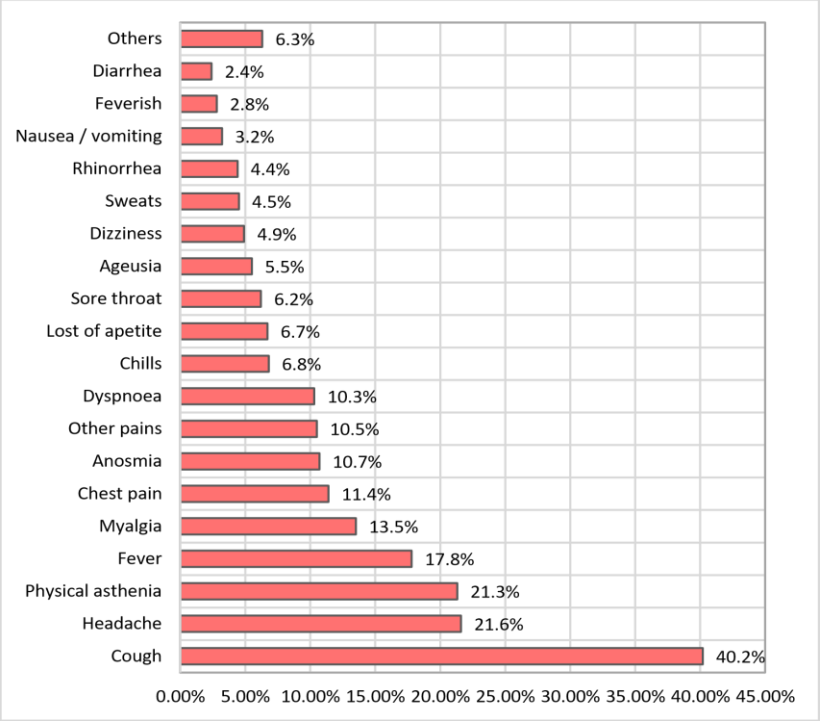

Fig. 1.4. The share of symptoms recorded in the batch

Table 1.I. Distribution of recorded symptoms, compared by gender and age range

Symptoms	Sex M		Sex F		*p*	Age range				*p*
						under 60 years		over 60 years		
	n	%	n	%		n	%	n	%	
Rhinorrhea	21	5.4%	13	3.3%	,145	32	6.0%	2	0.8%	.001**
Dyspnoea	46	11.9%	34	8.7%	,133	49	9.3%	31	12.4%	,178
Chest pains	49	12.7%	40	10.2%	,270	62	11.7%	27	10.8%	,706
Cough	157	40.7%	156	39.7%	,781	202	38.2%	111	44.4%	.099
Fever	84	21.8%	55	14.0%	.005**	90	17.0%	49	19.6%	,379
feverish	9	2.3%	13	3.3%	,411	18	3.4%	4	1.6%	,156
Chills	26	6.7%	27	6.9%	,941	37	7.0%	16	6.4%	,758
Sweats	20	5.2%	15	3.8%	,358	2. 3	4.3%	12	4.8%	,776
myalgia	49	12.7%	56	14.2%	,525	80	15.1%	25	10.0%	,051
sore throat	15	3.9%	33	8.4%	.009**	40	7.6%	8	3.2%	,018*
anosmia	29	7.5%	54	13.7%	.005**	70	13.2%	13	5.2%	.001**
ageusia	18	4.7%	25	6.4%	,299	35	6.6%	8	3.2%	,051
Physical weakness	65	16.8%	101	25.7%	.003**	89	16.8%	77	30.8%	,000**
dizziness	15	3.9%	2. 3	5.9%	,203	19	3.6%	19	7.6%	.015*
DISORDERS	80	20.7%	88	22.4%	,572	134	25.3%	34	13.6%	,000**
loss of appetite	2. 3	6.0%	29	7.4%	,427	27	5.1%	25	10.0%	,011*
Nausea / vomiting	9	2.3%	16	4.1%	,168	16	3.0%	9	3.6%	,671
Diarrhea	4	1.0%	15	3.8%	,012*	10	1.9%	9	3.6%	,149
Other pains	39	10.1%	43	10.9%	,703	39	7.4%	43	17.2%	,000**
Other	24	6.2%	25	6.4%	,934	27	5.1%	22	8.8%	.047*
Asymptomatic	99	25.6%	106	27.0%	,675	152	28.7%	53	21.2%	.026*

They did not register a distinction statistically significant neither between sexes, neither between the elderly patients and the others .It is possible however to notice that in men they are slightly common than in women, cases with 1-4 symptoms (70.4% versus 62.9%), in time what about women are fairly common cases with 5-9 symptoms (10.3% vs. 3.8%); in patients under 60 they are more common cases with 5-9 symptoms (7.3% versus 6.4%), and in patients over 60 there are common the cases with 1-4 symptoms (72.4% versus 63.9%).

In patients with comorbidities, significantly increased percentages of cough (43.2% versus 34.8%), physical asthenia (25.6% versus 13.6%), loss of appetite (8.4% versus 3 .6%), pains other than chest pain (12.8% versus 6.5%) and other sporadic symptoms (8.2% versus 2.9%) – tachycardia, dysphagia, insomnia, psycho motor agitation, paresthesias, jaundice, nitrogen retention syndrome and even syncope (2 cases). Patients without comorbidities present significantly more frequently anosmia (15.4% vs. 8.0%), odynophagia (8.6% vs. 4.8%), and rhinorrhea (7.5% vs. 2.6%); as expected, the percentage of asymptomatic patients

is also slightly higher among those without comorbidities (29.7%) compared to others (24.4%) – Table 1.II.

Table 1.II. Distribution of recorded symptoms, compared to patients with/without comorbidities

	Comorbidities									
	ABSENCES		present		p		ABSENCES		present		p
	n	%	n	%			n	%	n	%	
Rhinorrhea	21	7.5%	13	2.6%	.001**	anosmia	43	15.4%	40	8.0%	.001**
Dyspnoea	22	7.9%	58	11.6%	,102	ageusia	18	6.5%	25	5.0%	,395
Chest pains	34	12.2%	55	11.0%	,618	Physical weakness	38	13.6%	128	25.6%	,000**
Cough	97	34.8%	216	43.2%	,021*	dizziness	10	3.6%	28	5.6%	,210
Fever	50	17.9%	89	17.8%	,966	DISORDERS	66	23.7%	102	20.4%	,289
feverish	12	4.3%	10	2.0%	,063	loss of appetite	10	3.6%	42	8.4%	,010*
Chills	21	7.5%	32	6.4%	,549	Nausea / vomiting	6	2.2%	19	3.8%	,210
Sweats	11	3.9%	24	4.8%	,580	Diarrhea	7	2.5%	12	2.4%	,925
myalgia	41	14.7%	64	12.8%	,458	Other pains	18	6.5%	64	12.8%	.006**
sore throat	24	8.6%	24	4.8%	.034*	Other	8	2.9%	41	8.2%	.003**
						Asymptomatic	83	29.7%	122	24.4%	,104

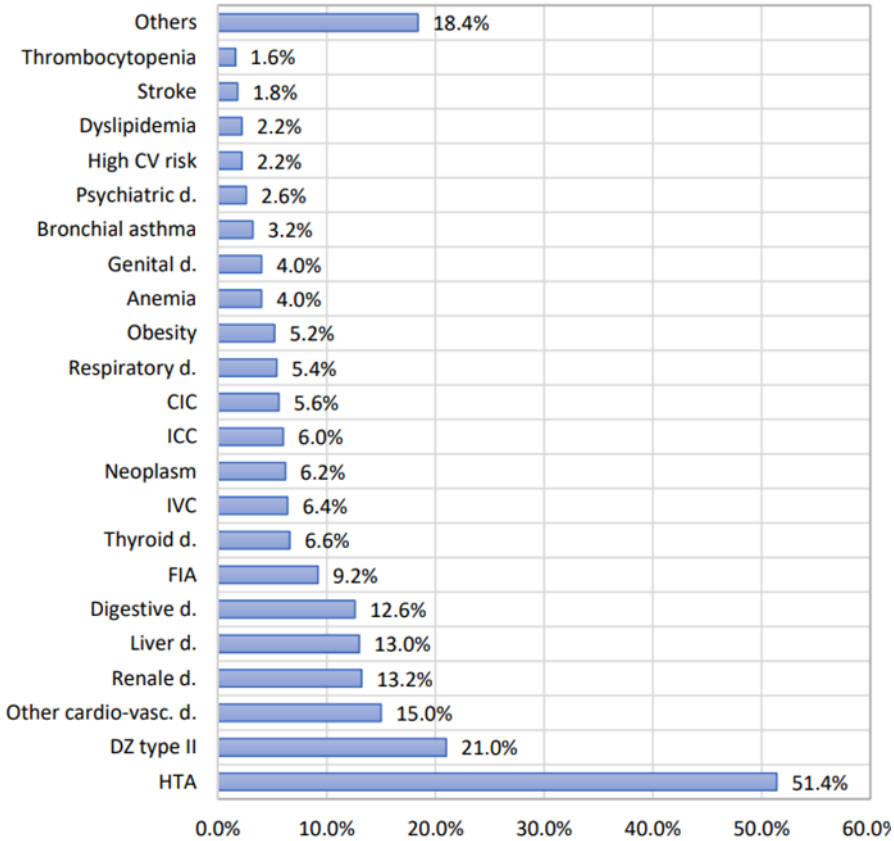

Fig. 1.5. Distribution of comorbidities in the study group

Among the investigated patients, 500 (64.2%) presented various comorbidities, the most frequently recorded being HTN (51.4%) and DM type II (21.0%). A quarter of the patients (25.0%) presented a single comorbidity, 17.3% presented 2 comorbidities, 12.3% presented 3 comorbidities and 9.5% presented 4-7 comorbidities - Figure 1.5.

No statistically significant differences are observed between patients with comorbidities and others regarding the classification of symptoms according to their number; among patients with comorbidities, cases with 1-4 symptoms are slightly more frequent (68.6% versus 63.1%), cases with 5-9 symptoms being noticed in similar percentages both in them (7.0%) and in those without comorbidities (7.2%).

86.5% of patients (674) were examined with lung CT, to identify possible complications; 125 patients (18.5%) showed no changes. In the others, the ground glass appearance was most frequently observed (59.9%) and that of accentuated interstitial drawing (8.9%), along with condensation (7.1%), fibronodular sequelae (7.1%) and confluence (6.7%) – Figure 1.6.

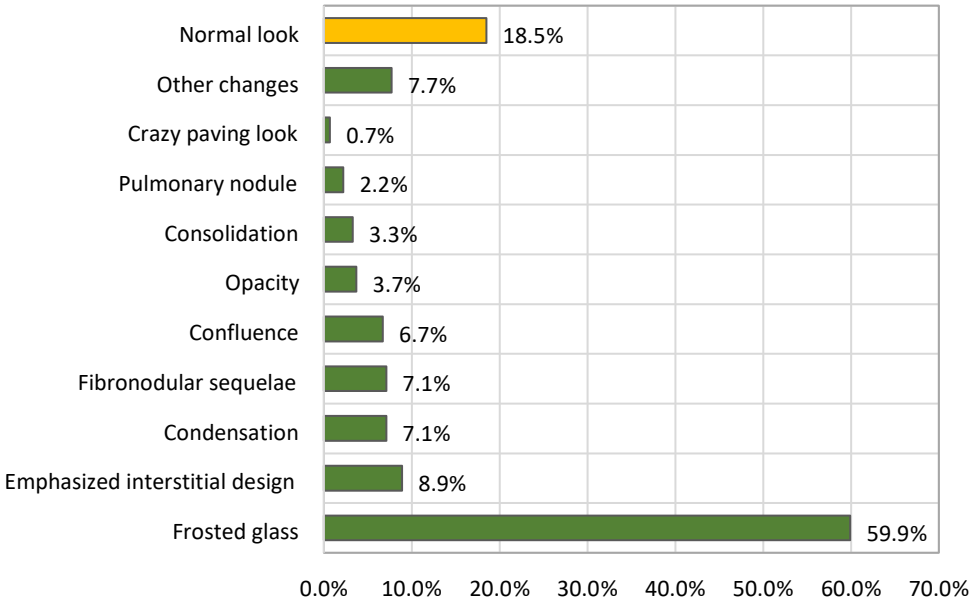

Fig. 1.6. Distribution of CT changes in the study group

There are no statistically significant age, compared to only 12.8% of those with differences between the sexes regarding the normal appearance; therefore age over 60 proportion of patients with modified CT; years has an associated risk OR = 4.123 of instead, 37.7% of them are over 60 years of causing pulmonary complications.

We compared the presence of symptoms and comorbidities in patients with normal and modified CT, in order to identify possible correlations; we also included in the study patient gender and age group, which may also have significant influences. In cases where we identified statistically significant differences in the weight of symptoms in patients with modified CT compared to others, we also calculated the risks associated with the respective symptoms, OR (Odds Ratio).

Symptoms present in significantly increased percentages in patients with modified CT compared to others were chest pain, cough, fever, physical asthenia and loss of appetite; among these, the highest associated risk is found for inappetence (OR = 6.027), followed by physical asthenia (OR = 2.809) and cough (OR = 2.342). It also found that 41.6% of patients with normal CT were asymptomatic, compared to only 21.1% of those with altered CT – a difference that was also statistically significant and associated with symptoms acts as a factor protection for subunit risk, meaning that the absence of lung changes.

We identified statistically significant differences between patients with normal CT and those with pulmonary changes in the number of symptoms; thus, 71.1% of patients with

modified CT have 1-4 symptoms, and of these, 31.0% have 3 symptoms; on the other hand, only 53.6% of patients with normal CT have 1-4 symptoms, and only 14.4% have 3 symptoms. Also, 7.8% of patients with modified CT have 5-9 symptoms, compared to 4.8% of those with normal CT. Distribution symptoms taking into account their number, compared to patients with / without CT with altered appearance.

The ROC analysis for the number of symptoms reveals an AUC coefficient (Area under Curve) of 0.622, with a confidence interval between 0.565-0.678, statistically significant (p = .000**), but still indicating a rather poor discriminatory power for identifying lung changes. The identified cut - off value is 1.50, corresponding to a sensitivity of 0.681 and a specificity of 0.528 (Figure 1.7) – therefore it can be considered that the risk of pulmonary changes increases in patients with more cumulative symptoms, compared to those with only one symptom.

The percentage of patients with comorbidities among those with lung changes (69.8%) is statistically significantly higher than the similar percentage among patients with normal lung appearance (47.2%). From the list of monitored comorbidities, statistically significant differences were recorded between patients with altered lung appearance and those with normal lung appearance in the case of HTN (37.0% vs. 16.0%), AIF (7.5% vs. 0.8%).

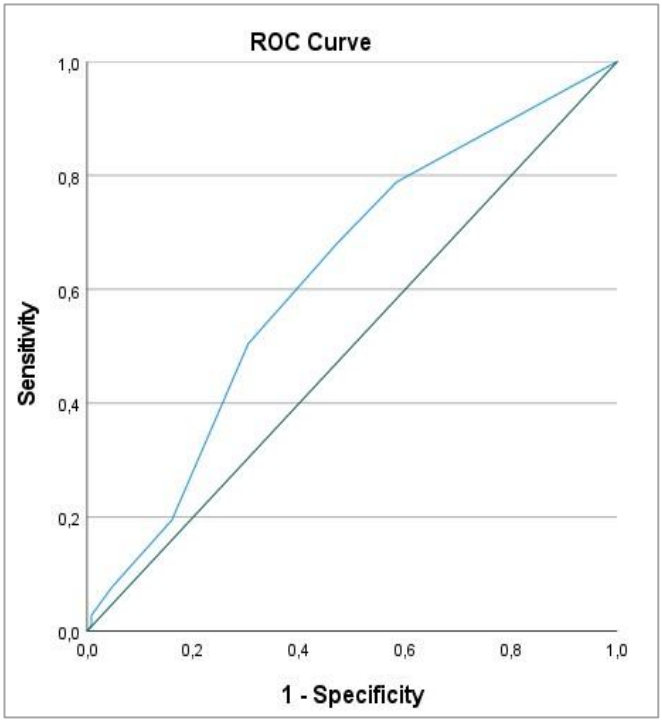

Fig. 1.7. No. symptoms vs. CT change – ROC curve

Type 2 DM (16.4% vs. 2.4%), renal disease (9.1% vs. 3.2%), and psychiatric disease (1.3% vs. 4.8%). The risk calculation shows that, in general, the presence of comorbidities is associated with an important risk of pulmonary changes, OR = 2.581, and among the comorbidities, the highest risk corresponds to AIF (OR = 10.008), followed by DM type 2 (OR = 7.974) and respectively HTN (OR = 3.080) and kidney diseases (OR = 3.031), with similar risks; the presence of psychiatric conditions is not relevant for pulmonary changes, the recorded risk being sub-unit.

Conclusions

There are certain correlations between the symptoms noticed in patients, their comorbidities and the appearance of lung changes on CT, respectively febrile or afebrile evolution. Among the characteristic symptoms of COVID-19, patients with comorbidities

present more frequently cough, physical asthenia, inappetence and other than chest pain compared to those without comorbidities. Also, the symptoms present in significantly increased percentages in patients with modified CT compared to the others were chest pain, cough, fever, physical asthenia and loss of appetite, and the characteristic comorbidities were HTN, AIF, DM type II and renal diseases; however, the multivariate analysis identifies 4 significant predictors for the occurrence of pulmonary complications, namely age over 60 years, the presence of cough, at least 2 cumulative symptoms and type II diabetes.

I.1.2.2. The relationship between biochemistry changes and antibiotic treatment - a bridge from molecular mechanisms to clinical signs

I.1.2.2.1. Molecular changes in the oral microbiome

Introduction

The oral cavity presents one of the most concentrated and various microbial populations. The oral cavity hosts a complex microbial ecosystem with different species and development particularities according to the anatomic structure (lips, teeth, tongue, jugal mucosa, palate, saliva, gingival cleft) and the artificial constructions they are located on (bridges, dental prostheses). Numerous bacterial species interact either in a synergetic manner, creating the proper environment or the necessary food for the survival of others, or antagonistic manner - some species are in competition with the others for food and survival (Solovan et al, 2006; Duceac et al, 2018; Duceac et al, 2018).

Oral microbial flora presents itself as follows: Gram positive bacilli, Gram negative bacilli, cocci or aerobic and anaerobic bacilli. Anaerobic or optional anaerobic streptococci represent almost 80% of the total viable germs (Schwiertz et al, 2016; Scutariu et al, 2016). Candida and coliforms are indigenous in the oral cavity of adults. Protozoans are present in a smaller number; in high number indicate a poor oral hygiene. Lactobacilli are found near the cavities. Candida is much more frequent in patients who were subjected to a treatment with antibiotics than in those who were not treated (Botnariu et al, 2018; Raftu et al, 2018; Sherwood et al, 2013).

The factors influencing the development of oral microbial flora are: the oral environment which creates the favourable conditions for bacterial species to survive and reproduce: humidity, neutral pH, food, and as long as these features of the environment are present, the bacteria will continue to exist; adherence to the epithelial cells of the mucosa, dental enamel and dentin form intergeneric coaggregates; protection areas are those places that protect the poorly adherent microbial species: occlusion fossette, enamel fissures, polysaccharidic matrix of the pellicle acquired from the surface of the dental hard tissue, gingival sulcus; the elimination of the microorganisms from the oral environment occurs naturally through the desquamation of the oral epithelium, the salivary flux, movements of the tongue and soft tissue, through mastication and deglutition and artificially by tooth brushing, use of dental floss and mouthwater; the nutrients necessary for the survival of bacteria come from food, being mainly carbohydrates and saccharides which through metabolization by part of certain microbial species from the bacterial plaque that adheres to the hard intraoral structures decrease the pH level and initiate the demineralisation process of the enamel; local or systemic antimicrobial therapy (antibiotics) affect the balance of the oral flora favouring the proliferation of fungi, involved in the cutaneous-mucous infections (Rogers et al, 2008; Ciurcanu et al, 2016; Roman et al, 2015).

Material and methods

The study includes 127 patients with bacterial infections, studied in the interval 2014-2018. The group of etiological factors cause infectious diseases. An infectious disease or

infection must be seen as the ensemble of phenomena that take place in the organism due to the presence, proliferation and the action of microorganisms. In an infectious process there are important: the microorganism (the pathogenic agent of the infection), the macroorganism (where the germ conducts its biological activity) and the external environment which exerts its influence on the features of both macro- and microorganisms. Infectious diseases are not caused by just any type of germ. Infections can be caused by certain species of germs characterized by pathogenicity. Humans can be carriers of pathogenic germs without getting ill. In the oral cavity of certain people can be discovered pathogenic bacilli of diphtheria or meningococci, but nevertheless, these individuals are not ill of diphtheria or meningitis (healthy germ carriers). The explanation resides in the different causes that are connected with the features of pathogenic germs and with the resistance of that particular organism. Although the representative species for the microbial flora can be isolated from most of the areas of the oral cavity, certain surfaces – tongue, dental surface, gums, saliva – tend to favour the preferential colonisation with certain specific microorganisms.

Results

Based on the clinical signs of the patient, the clinician should be suspicious about the apparition of bacteraemia and choose the right moment for taking the sample for hemoculture. The diagnostic algorithms implemented by different researchers are intended to help the clinician in managing the great amount of clinical data and in transforming this information in predictive scores.

The treatment with antibiotics has only a therapeutic purpose in the basic treatment of post-surgical infection, as helping treatment in surgical infections (abscess, infection of salivary glands), but also in the prophylaxis of superinfection.

The oral cavity hosts a complex microbial ecosystem that includes different species and development particularities depending of the anatomical structure (lips, teeth, tongue, jugal mucosa, palate, saliva, gingival cleft) or artificial constructions on which they locate (bridges, dental prostheses). The numerous bacterial species interact in a synergetic manner, that is they help each other, some create the proper environment.

The normal oral flora contributes at the protection against infection by: producing bactericidal substances; producing Ig A and peroxidase which interact with the thiocyanate ions from food and the hydrogen-peroxidase produced by the commensal flora; producing lysozyme and lactoferrin; the existence of salivary proteins can inhibit, at their turn, the adherence of bacteria on the surface of teeth and the oral mucosa; the quick turnover from the level of the oral epithelium also helps removing the bacteria that adhered at this level.

Gingival inflammation can be associated to bacterial infection. When the pulp is inflamed it results a constant pressure on the dental nerves and the neighbouring tissues. The pressure can generate moderate or extreme pain, depending of the degree of inflammation and the organism's immunity. The bacterial plaque can be differentiated into two main types, depending of the place where it is formed. Thus, there is a supragingival bacterial plaque (appears on the surface of the teeth and on the oral mucosa, roof of the mouth and the tongue) and the subgingival bacterial plaque, situated in the gingival sulcus and the periodontal pocket. When the plaque is thick enough it can be easily noticed with the naked eye.

The formation mechanism of the dental plaque includes: absorption of proteins and bacteria which form a thin film on the surface of the teeth; the adhesion of bacteria to the already formed film; the irreversible adhesion of bacteria due to the intermolecular interaction between the pellicle and the dental cells; the secondary colonizing bacteria attach to the primary ones; cells divide resulting a biofilm.

The initial phase of this mechanism lasts almost 2 hours, while the surface of the teeth and the mucosa of the oral cavity are invaded by salivary proteins, food remnants and cellular

residues. The initial film continues being populated by secondary bacteria and developing and turning into mature bacterial plaque (in almost 30 days).

The causes leading to the offset of the mechanism forming the bacterial plaque are the quantity and the quality of the saliva, diet, age, daily habits of dental hygiene and the eventual secondary disorders of the organism which can increase the predisposition to the formation of dental plaque and tartar.

Discussions

The bacterial plaque consists of numerous microorganisms as streptococci (*Streptococcus mutans, Streptococcus salivarius, Streptococcus sagitus, Streptococcus mitis, Lactobacillus*), spirochetes or protozoans (Cuciureanu et al, 2019; Fine et al, 2008).

Dental plaque causes cavities when the acids from the oral cavity affect the enamel of teeth. When not removed, it can cause irritations of the gum and gingivitis, periodontal diseases and even tooth loss (Cuciureanu et al, 2019; Fine et al, 2008).

Primary dental plaque can be removed through the correct brushing of teeth; it helps eliminating the resulting film and the soft deposits from the surface of teeth and gums. Tooth brushing is recommended after every meal, in the morning and in the evening (Ljungh et al, 2009; Ciurcanu et al, 2016; Hinganu et al, 2018).

The use of dental floss is also important for the effective elimination of the bacterial plaque positioned between the teeth (where the toothbrush has no access). Bacterial plaque can also be effectively eliminated using oral irrigators, gum stimulators, interdental brushes and special devices for tongue cleaning (Ljungh et al, 2009; Ciurcanu et al, 2016; Hinganu et al, 2018).

The energy of the laser can have a significant effect on the microbial flora by deforming the walls of cells; a thermic effect by attracting the pigment in the cells of specific microorganisms as *Porphyromonas gingivalis* and a direct effect of thermic heat (Willey et al, 2014; Rickard et al, 2008; Hajishengallis et al, 2008).

Consequently, we must consider the tooth that needs to be extracted is a reservoir of infection and not necessarily the instruments. Hence, when the physician estimates a high microbial load after the extraction, he recommends an antibiotic treatment (Willey et al, 2014; Rickard et al, 2008; Hajishengallis et al, 2008).

For maintaining the optimal state of dental hygiene it is recommended to perform a complete professional hygienization with tartar removal and airflow with bicarbonate, at least once a year.

The use of mouthwash twice a day helps maintaining teeth and gums healthier even in the areas where access is more difficult. Moreover, it prevents and reduces dental plaque, one of the gingival causes, and strengthens the enamel of teeth.

Conclusions

The microorganisms from the oral fluid are different from those that live on the hard tissues forming the bacterial plaque; they are more vulnerable and easier to remove by means of oral hygiene unlike the ones forming the bacterial plaque adherent to the teeth and which are more resistant and more difficult to remove.

Maintaining the balance of the oral microbial ecosystem is essential because commensal bacteria have a protective role, helping the immune response and preventing the development of other pathogenic species that make the organism ill.

CHAPTER 2: RESEARCH CONTRIBUTIONS IN METABOLIC DISEASES

I.2.1. STATE OF THE ART

Metabolic syndrome (MetS) represents a cluster of metabolic abnormalities that include hypertension, central obesity, insulin resistance, and atherogenic dyslipidemia, and is strongly associated with an increased risk for developing diabetes and atherosclerotic and nonatherosclerotic cardiovascular disease (CVD). The pathogenesis of MetS involves both genetic and acquired factors that contribute to the final pathway of inflammation that leads to CVD. MetS has gained significant importance recently due to the exponential increase in obesity worldwide (Rochlani et al, 2017).

Metabolic-associated fatty liver disease is diagnosed based on the finding of hepatic steatosis (seen in diagnostic imaging, elastography or histology), as well as type 2 diabetes mellitus and/or overweight and/or obesity and/or hyperlipidaemia, regardless of alcohol consumption.

It is already known the relationship between of diabetes mellitus and non-alcoholic fatty liver disease (NAFLD). NAFLD comprise a large spectrum of disordes, hepatic steatosis, inflammation, cirrhosis, fibrosis and hepatocarcinoma (Figure 2.1) (Powell et al, 2021).

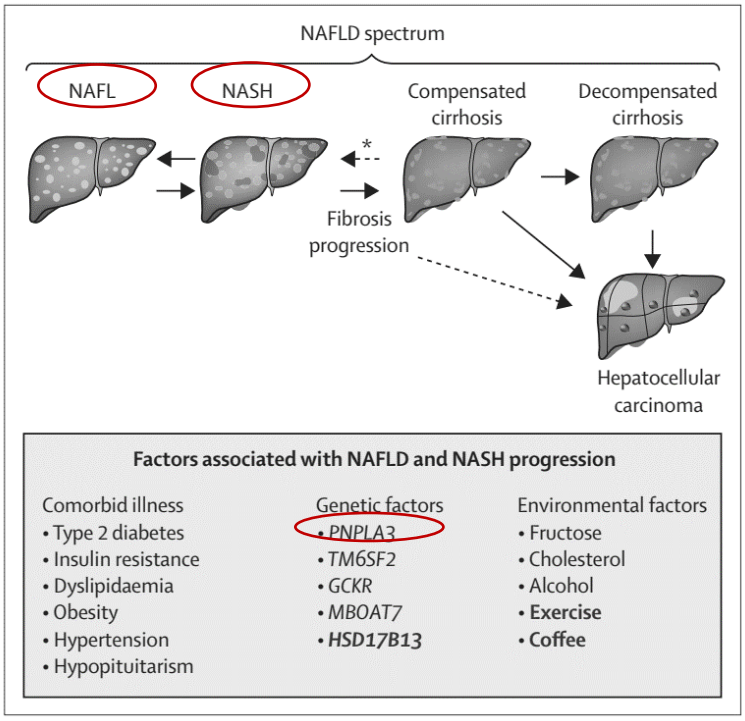

Fig. 2.1. Spectrum of NAFLD (Adapted after *Powell et al*, 2021)

Over the past four decades, NAFLD has become the most common chronic liver disorder (with a global prevalence of around 25% of the adult population) (Matteoni et al, 1999) and is recognised to have a close, bidirectional association with components of metabolic syndrome (Rinella et al, 2016). Although less than 10% of people with NAFLD develop liver-related complications, a key challenge is to identify those who are at the highest risk among the many people affected by NAFLD. Due to its high prevalence, NAFLD is now the most rapidly increasing cause of liver-related mortality worldwide (Younossi et al, 2016) and is emerging as an important cause of end-stage liver disease (Younossi et al, 2019), primary liver cancer (Sima et al, 2014), and liver transplantation with

a substantial health economic burden. Despite the growing concern, NAFLD is underappreciated as an important chronic disease6 and there are few national strategies or policies for NAFLD (Powell et al, 2021).

Although only a small part percentage of patients with hepatic steatosis will develop severe hepatic disorders, subjects with type 2 diabetes mellitus present a higher risk for hepatocarcinoma. The hepatic accumulation of lipids seems to be an essential process in NAFLD physiopathology (Romeo et al, 2020). In 2008, a polymorphism of the PNPLA3 gene (patatin-like phospholipase domain-containing 3) was reported to be a determinant genetic factor of NAFLD (Romeo et al, 2008). PNPLA3, also called adiponutrin or calcium-independent phospholipase A2-epsilon, is part of a family of proteins with lipase/transacetylase activity (Kienesberger et al, 2009). PNPLA3 has been proved to play a significant role in determining the fatty hepatic load independent of obesity.

Cardiovascular disease represents the principal cause of death and morbidity among people with diabetes, especially in those with type 2 diabetes mellitus. Adults with diabetes have 2-4 times increased cardiovascular risk compared with adults without diabetes, and the risk rises with worsening glycaemic control. Diabetes-related macrovascular and microvascular complications, including coronary heart disease, cerebrovascular disease, heart failure, peripheral vascular disease, chronic renal disease, diabetic retinopathy and cardiovascular autonomic neuropathy are responsible for the impaired quality of life, disability and premature death associated with diabetes (Dal Canto et al, 2019).

Diabetes mellitus due to changes in glycaemic status causes multi-organ damage.and that is why it became a current health problem with a growing incidence and prevalence, younger ages being affected as well. It is a chronic condition that concerns the state of nutrition of the whole organism and through the development of which disrupts the progress of the intermediate metabolism of all the food principles: carbohydrates, lipids, proteins (Mohamed et al, 2016; Danaei et al, 2011; Reid et al, 2006; Levinthal et al, 1999).

The oral cavity, also called the body's mirror, has been a concern since ancient times. “In the Roman Law of the Twelve Tables“, c 450BC, natural teeth were valued, it states: 'Whoever shall cause the tooth of a free man to be knocked out shall pay a fine of three hundred as’. The Romans (c 25BC – c 50AD) were the first recommending that city dwellers should wash their mouths out in the morning” (Mohamed et al, 2016).

A large number of studies have evaluated both the incidence of lesions in the oral cavity and the interrelationship between this disease and various avitaminosis with repercussions on the oral cavity. The most concentrated and various microbial populations are in the oral cavity that hosts a complex microbial ecosystem with different species and development particularities according to the anatomic structure (lips, teeth, tongue ,jugal mucosa, palate, saliva, gingival cleft) and the artificial constructions they are located on (bridges, dental prostheses). The bacterial species interact either in a synergetic manner, creating the proper environment or the necessary food for the survival of others, or antagonistic manner - some species are in competition with the others for food and survival (Mohamed et al, 2016; Danaei et al, 2011; Reid et al, 2006; Levinthal et al, 1999).

It is known the fact that hyperglycaemia of the diabetic patient is incriminated in altering the microbial flora of the oral cavity. Among the mechanisms involved in the occurrence of periodontitis, the frequent disease of the oral cavity in diabetic patients, there is the activity of inflammatory markers along with epigenetic modifications in the regulation of gene expression.

Another problem linked with Diabetes is regarding the incidence of malformed children born to diabetic mothers. More than 30% of unexplained congenital malformations are due to diabetes or maternal prediabetes. The pathogenic mechanism of diabetic malformations is still unknown. The syndrome finds two children with malformations from

45 (4.4%) children born to pre-diabetic mothers 5 years before the appearance of diabetes itself and a good glycaemic balance is the best guarantee that pregnancy will develop normally (Mohamed et al, 2016).

I.2.2. SCIENTIFIC CONTRIBUTIONS

The relationship between the presence of metabolic syndrome and the development of type 2 diabetes and cardiovascular disease has been amply demonstrated. The interest in metabolic syndrome and visceral obesity is renewed because it has been linked to other chronic diseases, such as dementia and some cancers. Lifestyle changes (in particular, reduction of body weight through diet, exercise, including salt restriction) remain the most important strategies in metabolic syndrome management by decreasing arterial tension and improving metabolic abnormalities. Further studies on new drugs and devices to address the complexity of the mechanisms involved in the emergence of metabolic syndrome are needed. In this sense, I was concerned with researching the implications of diabetes at the hepatic, cardiovascular, oral and gestational level.

The main preoccupation that I had in this direction of research was materialized in the next scientific papers:

Published articles

1. The Influence of Metabolic Factors in Patients with Chronic Viral Hepatitis C Who Received Oral Antiviral Treatment, Gavril OI, Gavril RS, Mitu F, Gavrilescu O, Popa IV, Tatarciuc D, Drugescu A, Oprescu AC, Gherasim A, Mihalache L, **Esanu IM**. Metabolites, 2023, 13(4): art. no 571. IF=4.1
2. Correlations between PNPLA3 gene polymorphisms and NAFLD in type 2 diabetic patients, Gavril OI, Arhire LI, Gavril RS, Zota MI, Gherasim A, Nita O, Drugescu A, Oprescu AC, **Esanu IM**, Mitu F, Graur M, Mihalache L. Medicina (Kaunas) 2021; 57(11):1249. doi: 10.3390/medicina57111249. PMID: 34833467; PMCID: PMC8620174.
3. The role played by novel inflammatory markers in assessment of peripheral artery disease, Onofrei V, Crișan A, Adam CA, Marcu DTM, Haba MȘC, Tribus LC, Ceasovschih A, **Eșanu IM**, Petroaie AD, Crișan-Dabija R, et al. Medicina. 2023; 59(9):1557. doi: 10.3390/medicina59091557.
4. Advantages of thoracic electrical bioimpedance used for hypertension control in metabolic syndrome patients, **Esanu IM**, Cotea I, Boanca M, Paraschiv C. Romanian Journal of Oral Rehabilitation 2012; 4(2):39-43 (Index Copernicus, DOAJ).
5. Cardiovascular risk amelioration in the metabolic syndrome – primary prophylaxis *vs.* secondary prophylaxis, **Esanu I**, Boanca M, Cotea I, Paraschiv C, Forna N. Romanian Journal of Oral Rehabilitation 2013, 5(4):13-21 (Index Copernicus, DOAJ).
6. Value of the Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio in Predicting CPET Performance in Patients with Stable CAD and Recent Elective PCI Drugescu A, Roca M, Zota IM, Costache A-D, Gavril OI, Gavril RS, Vasilcu TF, Mitu O, **Esanu IM**, Roca I-C, et al. Medicina. 2022; 58(6):814.
7. Influence of diabetes mellitus and smoking on pro and anti-inflammatory cytokine profiles in gingival crevicular fluid, Pasarin L, Martu MA, Ciurcanu OE, Luca EO, Salceanu M, Anton D, Martu S, **Esanu IM**. Diagnostics 2023
8. Risk factors for periodontal disease in diabetic patients, Paraschiv C, **Esanu I**, Gavrilescu CM, Ghiuru R, Munteanu D, Manea P. Romanian Journal of Oral Rehabilitation 2018; 10(3):199-204 (Web of Science Core Collection, DOAJ).

9. The role of chemical factors in the diabetes and the prediabetes that leads to polimorfe oro-maxilo-facial alterations in malformative syndrome, **Esanu I**, Constantin I, Budacu CC, Agop Forna D, Mihai C. Revista de Chimie 2019; 70(6):2112-2117. IF=1.605

10. Chemical factors which prompt oral pathological phenomena in some nutrition diseases, Lupusoru RV, Topor G, Miron IC, Grigore M, **Esanu I**. Revista de Chimie 2019; 70(5):1884-1887. IF=1.605

11. IL6 is correlated with metabolic syndrome parameters in oral lichen planus, Țăranu T, Constantin M, Ungureanu DA, **Eșanu IM**, Toader MP. 2015 E-Health and Bioengineering Conference (EHB), 1-4.

12. Colchicine in the treatment of refractory aphthous ulcerations: Review of the literature and two case reports. Toader MP, **Esanu IM**, Taranu T, Mocanu M, Toader Ș. Experimental and Therapeutic Medicine 2021; 21(3):281. https://doi.org/10.3892/etm.2021.9712

I.2.2.1. Liver disease and diabetes mellitus

In order to sustain my abilities in the field of liver examination techniques, I obtained the competence certificate in General Echography, by local contest-examination, in 2002, at "Grigore T. Popa" University of Medicine and Pharmacy Iasi coordinated by Prof. Carol Stanciu, Competence in General Echography (Certificate series 006608/29.05.2002), provided by Health Ministry, for Postgraduates doctors.

I.2.2.1.1. The influence of metabolic factors in patients with chronic viral hepatitis C who received oral antiviral treatment

Introduction

Global estimates indicate that 71 million people suffer from hepatopathies caused by chronic hepatitis C virus (HCV) infection. The prognosis and disease progression have significantly improved due to the availability of Direct-acting antiviral (DAA) therapy (Meryem et al, 2018; Kamp et al, 2019). The global accessibility of DAA treatment and the simplification of therapeutic strategies have made it possible to eliminate HCV by improving screening methods, identifying infected individuals with active infection, and initiating timely treatment. Regarding side effects related to other organs, antiviral medications may have a negative effect on the kidneys, cardiovascular system, and central nervous system. However, these side effects are rare and usually only occur in patients who already have serious comorbidities. It is important that patients are closely monitored during antiviral treatment so that any side effects can be identified and treated. Overall, however, oral antiviral treatment is considered safe and effective in treating hepatitis and has significant benefits for overall health (Rich et al, 2021).

The World Health Organization (WHO) estimates that elimination, as defined by them, can be achieved by up to 80% by 2030.Despite the significant breakthrough of DAA therapy in the treatment of HCV, with at least 95% of individuals achieving a sustained virologic response (SVR), the evolution remains uncertain due to the significant risk of hepatocellular carcinoma and the progression of hepatic disease. The severity of fibrosis and steatosis influences the post-SVR evolution and the possibility of hepatocellular carcinoma risk. HCV has been the leading cause of liver transplantation. However, it is currently being replaced by nonalcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma caused by toxic hepatic disease (Trotter et al, 2017; Gavril et al, 2021).

Epidemiological studies suggest a link between T2DM and HCV infection (Allison et al, 1994).

The oral antiviral treatment for patients with chronic viral C hepatitis is adapted according to current guidelines for this pathology (treatment for 8, 12, or 24 weeks depending on the severity of the disease). There are six genotypes and over 100 subtypes of HCV known. Among these, genotypes 1, 2, and 3 are found worldwide.

All subjects in our study presented genotype 1b, which predominates in Romania with a percentage of 99%.

Materials and Methods

We conducted a prospective study on 100 patients who had viral hepatitis C infection, including both newly diagnosed and previously known cases of liver disease. The study was conducted atthe Institute of Gastroenterology, "St. Spiridon" Hospital, Romania between 2018 (January) and 2020 (March). The subjects were investigated before and post SVR (after three months) based on imaging, biological (fasting blood tests), and clinical criteria such as upper gastrointestinal endoscopy andan abdominal ultrasound exam. The first evaluation occurred before antiviral treatment, while the second visit occurred three months post-SVR (T3). The DAA therapy consisted of either ombitasvir/paritaprevir/ritonavir + dasabuvir or ledipasvir + sofosbuvir. We considered chronic alcohol consumption subjects who consumed more than 100 g of alcohol per week.

The study enrolled patients who met the following inclusion criteria, in accordance with national and international guidelines: 18 years or older, with a positive RNA-HCV test, and who provided informed consent. Exclusion criteria included patients with undetectable HCV RNA, those who were not recommended for antiviral treatment because of their comorbidities, those with advanced forms of liver disease (indicated by clinical, biological, and imaging exams) such as hepatic encephalopathy, variceal gastrointestinal bleeding, ascites or jaundice, and those with hepatocellular carcinoma or other malignancies. All patients provided written informed consent before enrollment, and the research was conducted in agreement with the principles of the Declaration of Helsinki and received approval from the "Grigore T. Popa" University Ethics Committee, Iasi, Romania.

All patients who participated in the study were required to have 1b genotype with a complete liver panel to assess liver disease, detectable viremia, comorbidities evaluation, and approval from a specialist doctor for any other conditions. In addition, the degree of liver fibrosis was evaluated using a noninvasive test called Fibromax (the tests were sent to a certified laboratory—BioPredictive), both before treatment initiation and three months after achieving SVR. To ensure consistency in diagnosis, all enrolled patients underwent testing using the same method.

Fibromax is a blood test that requires a blood sample, minimum 2 mL, taken in the morning. Immunonephelometry and photometry were the techniques used to process the samples. Fibromax comprises five tests (noninvasive): FibroTest, which evaluates the liver fibrosis severity; ActiTest, which assesses the activity of necroinflammation; SteatoTest, which determines the hepatic steatosis degree; NashTest, which evaluatesifnonalcoholic steatohepatitis is present among metabolic syndrome subjects; and AshTest, which assesses the extent of liver damage in individuals who chronically consume alcohol (Terrault et al, 2018; Munteanu et al, 2018; Bril et al, 2018; Poynard et al, 2018).

Formulas correlated with date of birth, sex, height, and weight are used to calculate the results of the five noninvasive tests included in Fibromax: FibroTest, ActiTest, SteatoTest, NashTest, and AshTest. Blood tests including aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltranspeptidase, total bilirubin, fasting serum glucose, haptoglobin, alpha 2 macroglobulin, apolipoprotein A1, triglycerides and cholesterol are assessed using this score. For the current study, only the results of FibroTest and SteatoTest were taken into account. The local laboratory used a specific calibration for delimiting the degrees of liver fibrosis and steatosis based on the FibroTest and SteatoTest

scores. For instance, FibroTest score cut-offs for identifying the stages of liver fibrosis are $F4 > 0.75$, $0.5 < F3 \leq 0.75$, $0.25 < F2 \leq 0.5$, and $F1 \leq 0.25$, while SteatoTest score cut-offs for delimiting the stages for liver steatosis are $S3 > 0.75$, $0.5 < S2 \leq 0.75$, $0.25 < S1 \leq 0.5$, and $S0 \leq 0.25$.

The National Health Fund established the following criteria: patients with fibrosis levels F2, F3, and F4 were eligible for DAA initiation, while those with F0 and F1 fibrosis were not.

In order to conduct statistical analysis, version 18.0 of SPSS software was utilized. The data were analyzed descriptively using ANOVA. To compare frequency distributions within or between groups, the non-parametric Chi-Square and Kruskal–Wallis tests were employed. The normal range of values was assessed prior to applying tests for statistical significance, with continuous variables determined by the Skewness test. ANOVA test results provided information on mean value indicators (e.g., maximum and minimum values, median, mean, modulus) and dispersion indicators (e.g., standard deviation, standard error, coefficient of variation). To compare continuous variables across different groups, a significance threshold of 95% ($p < 0.05$) was used with Student's t-test and paired-samples t-test. Multiple comparisons of normally distributed value series were performed using a post-hoc Bonferroni test following one-way ANOVA.

Results

The study population comprised 100 individuals diagnosed with chronic hepatitis C infection, with 65% of them being female. The age range of the patients varied from 35 to 77 years. The mean was close to the median of the group, which indicated a uniform distribution of values, and this was corroborated by the Skewness and Kurtosis tests. Therefore, statistical tests of significance for continuous variables were applicable. The distribution of the participants by age and gender did not indicate any significant differences ($p = 0.089$). The treatment was administered to 72 patients with ombitasvir/paritaprevir/ritonavir + dasabuvir, while the remaining 28 received ledipasvir + sofosbuvir treatment.

The average value for fibrosis was 0.65 ± 0.18, ranging from 0.32 to 0.96, with a median value of 0.65, which suggested a homogenous distribution of the values and was supported by the Kurtosis and Skewness tests. As a result, tests for statistical significance were conducted for the continuous variables. Grade F4 fibrosis was observed in the majority of cases (43%) in the study.

The range of steatosis values was from 0.11 to 0.89, with a mean value of 0.50 ± 0.18, which was in proximity to the group's median value (0.49). Skewness and Kurtosis tests indicated a uniform distribution of values, which allowed for the use of statistical tests of significance for continuous variables. The majority of cases had grade S2 steatosis (37%). From the total study sample, 11% of patients were chronic alcohol consumers, 9.2% of women and 14.3% of men, with chronic alcohol consumption representing a slightly higher risk factor for males (RR = 1.35; 95% CI: 0.66–2.74; $p = 0.448$). Clinical parameter evolution in patients with fibrosis Regarding the evolution of laboratory parameters at a minimum of 3 months after achieving SVR, the following aspects were highlighted (Table 2.I.).

In patients with mild fibrosis:

Initially, the average BMI was significantly lower ($p = 0.043$), which remained so after SVR ($p = 0.034$); The average level of triglycerides ($p = 0.013$) significantly increased; Metabolites 2023, 13, 571 6 of 11 The average level of gamma-glutamyl transferase (GGT) ($p = 0.001$), alanine transaminase (ALT) ($p = 0.001$), and aspartate aminotransferase (AST) ($p = 0.001$) significantly decreased (to normalization).

In patients with severe fibrosis:

The mean levels of GGT (p = 0.001), AST (p = 0.001), and ALT (p = 0.001) decreased significantly. Evolution of clinical parameters in patients with steatosis. Regarding the evolution of laboratory markers post-treatment in patients with steatosis, the following aspects were highlighted (Table 2.II).

Patients with mild steatosis:

Initially, the mean BMI was significantly lower (p = 0.001), registering a significant increase after SVR (p = 0.004); The mean level of triglycerides (p = 0.001) significantly increased; The mean level of GGT (p = 0.005), ALT (p = 0.001), and AST (p = 0.001) significantly decreased (until normalization).

Patients with severe steatosis:

The mean level of GGT (p = 0.002), ALT (p = 0.001), and AST (p = 0.001) significantly decreased.

Table 2.I. Evolution of biological markers in patients with fibrosis

Parameter	T0	T3	Difference from the Average Period	*p* for Paired Sample T Test
BMI (kg/m^2) mild fibrosis severe fibrosis	25.96 ± 3.14 27.92 ± 5.14	27.18 ± 4.19 29.76 ± 4.66	+1.08 +1.64	0.410 0.183
***p* for F_{ANOVA} test**	0.043	0.034		-
Fasting blood glucose (mg/dL) mild fibrosis severe fibrosis	106.58 ± 37.23 116.72 ± 48.66	102.81 ± 27.43 113.52 ± 38.28	−3.78 −3.20	0.331 0.583
***p* for F_{ANOVA} test**	0.281	0.143		
Triglyceride (mg/dL) mild fibrosis severe fibrosis	105.28 ± 58.80 103.80 ± 35.74	126.17 ± 55.85 106.33 ± 39.23	+20.58 +2.53	0.013 0.617
***p* for F_{ANOVA} test**	0.833	0.039		
GGT (U/L) mild fibrosis severe fibrosis	38.18 ± 22.83 87.22 ± 77.35	22.47 ± 9.50 34.28 ± 25.34	−15.71 −52.94	0.001 0.001
***p* for F_{ANOVA} test**	0.001	0.010		
ALT (U/L) mild fibrosis severe fibrosis	74.24 ± 44.60 109.52 ± 75.46	26.64 ± 12.46 25.59 ± 15.05	−47.60 −83.93	0.001 0.001
***p* for F_{ANOVA} test**	0.012	0.724		
AST (U/L) mild fibrosis severe fibrosis	51.81 ± 28.64 86.81 ± 50.69	22.28 ± 6.79 24.63 ± 8.32	−29.53 −62.18	0.001 0.001
***p* for F_{ANOVA} test**	0.001	0.154		
LDL cholesterol (mg/dL) mild fibrosis severe fibrosis	123.83 ± 56.22 97.58 ± 32.49	125.67 ± 37.30 119.85 ± 38.66	+26.75 +16.30	0.175 0.504
***p* for F_{ANOVA} test**	0.269	0.585		-

Table 2.II. Evolution of biological markers in patients with steatosis.

Parameter	T0	T3	Difference from the Average Period	*p* for Paired Sample T Test
BMI (kg/m²) mild steatosis severe steatosis	25.39 ± 4.11 28.71 ± 4.49	29.62 ± 5.17 28.08 ± 4.05	+3.94 −0.84	0.004 0.457
***p* for F_{ANOVA} test**	0.001	0.198		-
Fasting blood glucose (mg/dL) mild steatosis severe steatosis	102.91 ± 32.02 115.58 ± 20.45	100.59 ± 26.80 118.68 ± 25.11	−2.32 +3.11	0.497 0.584
***p* for F_{ANOVA} test**	0.045	0.021		
Triglyceride (mg/dL) mild steatosis severe steatosis	88.89 ± 35.38 115.16 ± 44.45	106.00 ± 45.11 114.58 ± 46.11	+17.41 −0.58	0.001 0.950
***p* for F_{ANOVA} test**	0.001	0.148		
GGT(U/L) mild steatosis severe steatosis	40.20 ± 23.77 105.21 ± 95.67	24.02 ± 15.77 31.95 ± 17.51	−16.18 −73.26	0.005 0.002
***p* for F_{ANOVA} test**	0.001	0.011		
ALT(U/L) mild steatosis severe steatosis	58.64 ± 29.58 143.76 ± 69.84	23.27 ± 13.83 25.11 ± 9.22	−35.36 −118.66	0.001 0.001
***p* for F_{ANOVA} test**	0.001	0.090		
AST(U/L) mild steatosis severe steatosis	47.64 ± 17.07 108.46 ± 50.09	23.67 ± 10.14 23.26 ± 6.05	−23.98 −85.20	0.001 0.001
***p* for F_{ANOVA} test**	0.001	0.154		
LDL cholesterol(mg/dL) mild steatosis severe steatosis	98.75 ± 45.57 111.47 ± 44.89	119.48 ± 38.71 124.57 ± 37.65	+44.67 +5.48	0.064 0.692
***p* for F_{ANOVA} test**	0.636	0.623		-

T0, initial visit; T3, second visit; GGT, gamma-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL cholesterol, low-density lipoproteins cholesterol; BMI, body mass index.

Discussions

In our research, all the included subjects presented genotype 1b. These data are supported by other epidemiological studies that highlight the higher prevalence of genotype 1, followed by genotype 3 (Nahon et al, 2017). Studies show that a much higher prevalence of hepatic steatosis is encountered in HCV genotype 3 infections, compared to non-genotype 3 HCV (74% versus 48%) (Lonardo et al, 2006).

Our study aimed to evaluate the regression of fibrosis and steatosis severity using Fibromax (a noninvasive method), both before treatment and after achieving sustained virologic response (SVR) through DAA treatment. Previous studies have investigated this

relationship using hepatic elastography and percutaneous liver biopsy, with the latter being the preferred method for evaluating the severity of steatosis and fibrosis (Pan et al, 2018; Huang et al, 2020). Most patients with HCV who undergo DAA treatment achieve SVR (Laursen et al, 2020). Our study found that the majority of subjects, including those with mild and severe fibrosis, experienced regression of liver fibrosis. We also observed a significant decrease in the degree of hepatic fat accumulation.

The regression of liver fibrosis and steatosis remains a topic of debate, with some studies suggesting that the assessment method used plays a crucial role. Most studies use transient elastography to evaluate liver fibrosis (Rosso et al, 2020). However, in our study, we observed regression of fibrosis and steatosis in a substantial number of patients within a shorter time frame compared to other studies. This could be due to the use of Fibromax, which assesses liver function through biological parameters such as ALT, AST, and GGT. These values were found to change significantly three months after achieving SVR.

Despite our findings, it is important to note that our study has certain limitations. For instance, the sample size was small, and the monitoring period was short (9 months).

Additionally, liver fibrosis and steatosis severity were assessed non-invasively only with Fibromax (no other non-invasive methods, such as transient elastography, were used), rather than through direct histological examination. Furthermore, we did not account for other factors that could have influenced liver fibrosis and steatosis. Another limitation is that the use of concurrent medications that may affect liver steatosis (such as statins, pioglitazone, or vitamin E) was not collected. Moreover, not all the elements of metabolic syndrome were available. In another study, the prevalence of excessive alcohol consumption was 20% (Erman et al, 2019).

As observed in this study, the lowest BMI was found in patients with mild steatosis, and the highest in those with severe steatosis. These results are consistent with previous studies that report BMI and other anthropometric parameters factors associated with hepatic steatosis (Patton et al, 2004; Rubbia-Brandt et al, 2004; Mihalache et al, 2012). Moreover, regarding hepatic fibrosis, Ortiz et al. evaluated annual changes in the degree of hepatic fibrosis (rate of fibrosis progression) based on hepatic histology in HCV patients and highlighted that obesity is one of the main factors predicting disease progression (Ortiz et al, 2002).

In our study, similar findings were observed as in the literature, with the lowest BMI being more frequent in subjects with mild fibrosis and the highest in those with severe fibrosis. Weight loss can improve metabolic health markers and reduce the risk of developing diabetes and other metabolic conditions. BMI may play an important role in the progression of hepatitis C and treatment success, and maintaining a healthy weight after a viral cure may be important for long-term health improvement (Mukhtar et al, 2020; Nkwocha et al, 2022).

The pathogenesis of hepatic steatosis in patients with HCV infection is not precisely established, although it is associated with both viral and metabolic factors. Obesity is associated with insulin resistance in peripheral glucose, called insulin resistance, and can lead to the development of T2DM and contribute to the development of hepatic steatosis and cardiovascular (Sirinawasatien et al, 2020; Hurjui et al, 2012; Hurjui et al, 2012). Diabetes and obesity are well-known risk factors in the evolution of liver disorders (Shina et al, 2020; Lee et al 2022; Rhee et al 2019).

There is a clear association between triglyceride levels and chronic viral C hepatitis after treatment. In our study, a significant increase in triglyceride values was observed in both steatosis and hepatic fibrosis. Patients with hepatitis C may have elevated levels of triglycerides in the blood, and this may be associated with various metabolic conditions such as metabolic syndrome, nonalcoholic fatty liver disease, and diabetes mellitus. In patients

successfully treated for hepatitis C, triglyceride levels may decrease significantly, which can be a sign of metabolic improvement. However, it is essential to continue monitoring triglyceride levels and taking measures to maintain them within a normal range as high levels can represent a risk factor for health problems.

Risk stratification models for HCC have been developed among SVR patients using pre-treatment data. However, the time from obtaining SVR represents a complexity that has not yet been considered. In some patients with liver cirrhosis, the resolution of hepatic fibrosis and hepatic remodeling after SVR may lead to a decline in the risk of HCC over time. However, some subjects, especially those with decompensated HCV-related cirrhosis, may not exhibit a resolution of fibrosis and a decrease in HCC risk after SVR. As time passes after SVR, the patient's age increases and may acquire factors that decrease fibrosis or diminish the decrease in HCC risk after SVR (diabetes mellitus type 2, obesity, alcohol consumption), or even increase the risk of HCC among subjects without liver cirrhosis at the time of treatment.

Although the risk of HCC is significantly reduced after treatment with DAA, especially in patients with liver cirrhosis who achieve SVR, they still retain a high risk of HCC and thus require active surveillance. Biomarkers are necessary to identify those patients with the highest risk of HCC after a virologic cure. The optimal follow-up interval for patients with HCV after eradication treatment is not yet established.

Conclusions

After treatment for hepatitis C and achieving a viral cure, the risk of developing metabolic conditions may decrease. Our study showed a significant improvement in metabolic parameters in patients successfully treated with antiviral therapies. However, patients with a history of hepatitis C may still have an increased risk of developing diabetes and other metabolic conditions, even after a viral cure. Additionally, it is important to note that treatment for hepatitis C can be challenging for patients with pre-existing metabolic elements, as they may be more susceptible to adverse reactions and side effects of antiviral therapy.

I.2.2.1.2. Correlations between PNPLA3 gene polymorphisms and NAFLD in Type 2 diabetic patients

Introduction

Non-alcoholic fatty liver disease (NAFLD) comprises a large spectrum of disorders from simple fat loading of the liver (hepatic steatosis-defined by a hepatocytic fat loading of at least 5%) to hepatic inflammation (NASH-non-alcoholic steatohepatitis), fibrosis, and cirrhosis, with its well-known complication, hepatocarcinoma (Matteoni et al, 1999).

The hepatic accumulation of lipids seems to be an essential process in NAFLD physiopathology (Romeo et al, 2020). In 2008, a polymorphism of the PNPLA3 gene (patatin-like phospholipase domain-containing 3) was reported to be a determinant genetic factor of NAFLD (Romeo et al, 2018). PNPLA3, also called adiponutrin or calcium-independent phospholipase A2-epsilon, is part of a family of proteins with lipase/transacetylase activity (Kienesberger et al, 2009). PNPLA3 has been proved to play a significant role in determining the fatty hepatic load independent of obesity. Adiponutrin, a protein catalysing the hydrolysis of triglycerides, is expressed specifically at the hepatocyte level in the fatty tissue and suprarenal glands (Kotronen et al, 2009). Regarding the activity of PNPLA3 lipase on triglycerides and the activity of acylglycerol transacetylase, the gene expression is responsible for the lipase and for the accumulation of lipid droplets (Jenkins et al, 2004; Sookoian et al, 2012). Moreover, it is greatly influenced by nutritional stimuli at the transcriptional and posttranscriptional levels (Lake et al, 2005). Although several

potential regulating mechanisms exist regarding the deposition of hepatic lipids, one of them may be the PNPLA3 gene, which affects the remodelling of triglycerides (Chamoun et al, 2013; Ruhanen et al, 2014).

The association between alleles of PNPLA3 and the histological severity of hepatic disease has also been confirmed. Recent studies have shown that alleles of PNPLA3 are associated with an increased risk of hepatocarcinoma. Several studies have identified the fact that a common variant of the PNPLA3 gene (allele G rs738409) is strongly associated with NAFLD susceptibility and the degree of hepatic steatosis (Romeo et al, 2008; Kotronen et al, 2009; Sookoian et al, 2009). Importantly, in patients with the PNPLA3 GG genotype, the association between allele rs738409 G and NAFLD is present only in subjects under 50 years old. However, these findings were seen in one study which included only 162 subjects (Petta et al, 2013).

Genotyping for PNPLA3 could become part of the screening for patients with steatosis, as it might predict the risk for non-alcoholic steatohepatitis (NASH), hepatocellular carcinoma and cardiovascular disease.

In this study, the main interest is highlighting certain new relations between PNPLA3 genotypes and subclinical atherosclerosis for subjects with diabetes mellitus to confirm or invalidate the specificity of this gene for the stages of hepatic fatty disease as a marker or constituent of metabolic syndrome.

The objective of this study isto assess the relation between PNPLA3 genotypes and the degree of hepatic fatty loading in subjects with type 2 diabetes mellitus, as well as with the degree of subclinical atherosclerosis and components of metabolic syndrome and study the connection with the cardiovascular risk of this gene, viewed in the last few years as a marker of sensitivity regarding hepatic disease.

Material and methods

We performed an observational study on subjects with type 2 diabetes mellitus who did not receive insulin treatment, investigated in the Clinical Center for Diabetes, Nutrition and Metabolic Diseases of "Sf. Spiridon" Emergency Hospital Iași over a period of 18 months. The patients were evaluated in an outpatient-ambulatory setting. The inclusion criteria were as follows: type 2 diabetes mellitus treated with metformin and/or diet, subjects who signed the informed consent. The exclusion criteria were: subjects under insulin therapy, patients diagnosed with hepatitis B or C, toxic hepatitis, other hepatic conditions (Wilson's disease), pathological alcohol consumption (more than two units a day for men and one unit for women).

Body weight was measured in the morning, fasting, barefoot using a calibrated scale. Waist circumference was also assessed in the morning, at the end of a normal expiration at the approximate midpoint between the lower margin of the iliac crest and the last rib. Blood samples were collected in the morning, fasting.

The degree of liver fatty loading was assessed by ultrasonography using a portable ultrasound Carewell C12 with a convex probe of 3.5 MHz, and all subjects were assessed by the same examining physician. The followed parameters were liver to kidney contrast, parenchymal brightness, deep beam attenuation, bright vessel wall, and gallbladder wall definition (Hamaguchi et al, 2007).

Subclinical atherosclerosis was assessed using carotid intima-media thickness (CIMT) with a Doppler Color LS 128 ultrasound, with a linear probe of 7.5 MHz. The ultrasonographic exam was performed using B mode for both common carotid arteries. CIMT higher than 1 mm was considered abnormal, whereas values higher than 1.5mm were described as atheroma plaques (Randrianarisoa et al, 2015). The measurements were assessed by the same examining physician.

We assessed the components of metabolic syndrome according to International Diabetes Federation (IDF) criteria (Alberti et al, 2009):

- Waist circumference (WC)—normal values for WC were <80 cm in women and <94 cm in men; values exceeding these limits led to the diagnosis of abdominal obesity;
- Blood pressure—previous diagnosis of hypertension or values higher than 130 mmHg systolic blood pressure or higher than 85 mmHg diastolic blood pressure at the time of the clinical examination;
- Hypertriglyceridemia (triglycerides >150 mg/dL);
- Low values of high-density lipoprotein cholesterol (HDLc) (<40 mg/dL in men, <50 mg/dL in women);
- Hyperglycemia or type 2 diabetes mellitus (all the subjects met this criteria).
- The diagnosis of metabolic syndrome was made in the presence of three of the five disorders (Alberti et al, 2009).

Insulin sensitivity was measured using homeostatic model assessment (HOMA-IR) using the following formula: HOMA-IR = (fasting glucose × insulinemia)/22.5 (Matthews et al, 1985).

Genotyping of rs738409 was carried out by a high-resolution analysis of the dissociation of amplicons (HRM, high-resolution melting analysis) obtained by the amplification of a short genomic area that included the studied polymorphism. The HRM analysis was carried out with a Rotor-Gene 6000 instrument (Corbett Research, Australia).

For the purpose of obtaining amplicons containing the polymorphism site, two μL of genomic DNA extracted for standard genotyping was amplified with the help of 2 × SensiFastHRM master mix (Bioline, UK) and the primers GCCTTGGTATGTTCCTGCTTC and GGATAAGGCCACTGTAGAAGG were used at a final concentration of 200 nM. The thermal protocol applied was the activation of the enzyme for three minutes at 95 °C, followed by seven cycles of 10 s at 95 °C and 30 s at 67 °C, then 40 cycles of 10 s at 95 °C and 25 s at 60 °C. The length of the amplification process was 46 bp. The analysis of the dissociation curves was carried out using the device software (Rotor-Gene 6000 Series Software 1.7.87, Corbett Research, Australia). For the purpose of normalizing the curves, we selected a region of predissociation and one of postdissociation, inside which the relative fluorescence of each curve was considered to be 100% and 0%, respectively. The predissociation region was defined between 68.5 and 69.1 °C, while the postdissociation region was chosen between 77.0 and 78.6 °C.

In parallel with the genomic DNA samples, we analysed the following in each series of reactions:

- Three genotyping standards (CC, CG, GG), consisting of synthetic DNA molecules with a sequence that includes the genomic 46 bp region amplified with the help of the pair of primers used in the reaction;
- Three genotyping controls (rs738409 CC, CG, GG), consisting of genomic DNA sampled with a known PNPLA3 genotype; a negative amplification control, in which no DNA was introduced.

The statistical analysis was carried out using Statistica version 7.0 and SPSS v.20.The variables were described as mean ± standard deviation and with the 95%confidence interval for mean (if they were continuous variables) and as number and proportions (if they were discrete variables). When comparing average means between the two groups of continuous variables, the t-student test was performed (or the Mann–Whitney U test if the variances were not homogenous). For comparing more than two categories, for continuous variables, ANOVA test was performed (or Kruskal–Wallis test for non-homogenous variables). In ANOVA, if the statistical significance was obtained, post-hoc analysis was performed (Bonferroni test for homogenous variables or Tamhane test for non-homogenous variables)

to identify the significant differences between categories. For comparing discrete variables, Chi square ($\chi 2$) was used (for $\chi 2$ the significance threshold was $p = 0.1$). We considered $p < 0.05$ indicative of statistical significance. Logistical regression was performed to identify independent predictors for NAFDL.

This research was conducted in accordance with the Declaration of Helsinki and had the ethical approval of the "Grigore T. Popa" University of Medicine and Pharmacy (no 17140, 3 August 2016). All participants signed informed consent before entering the study.

Results

Among the 92 patients (44 men, 48 women) with type 2 diabetes mellitus investigated, 68 (73.91%) were from an urban environment, and 24 subjects (26.09%) were from a rural environment. The average age of the group was 60.38 ± 10.37 years, varying from 33 to 86 years of age.

While hepatic fatty loading was absent in 9.8% of the subjects (9 patients), 26.1% of the cases presented mild steatosis (24 patients), 36.9% presented moderate steatosis (34 patients), and 27.2% presented severe steatosis (25 patients). HOMA-IR was found to be significantly higher in patients with severe steatosis, compared to all other categories and also CIMT was significantly higher in patients with moderate steatosis compared to those with mild steatosis. When dividing the patients in two groups, those with no or mild steatosis, and those with moderate or severe steatosis, we confirmed that both HOMA-IR and CIMT were significantly higher in those with moderate or severe steatosis ($p < 0.001$, $p = 0.003$ respectively).

Moreover, metabolic syndrome was found in a significantly higher proportion in patients with moderate or severe steatosis (89.83% compared to 66.67%, $p = 0.008$). CIMT was above the normal limit (1mm) in 62% of the patients. Using binary logistic regression, we were able to show that steatosis associated with CIMT independently of age, sex, BMI, and WC.

Most of the included subjects (81.51%) met the criteria for metabolic syndrome.

We performed the genetic analysis on a subgroup of 68 patients, which maintained the same general characteristics as the study population. The CC genotype was the most common in the group we studied, with no statistical differences between men and women ($p = 0.297$) and was significantly more frequent in the group of subjects with severe steatosis (73.68% compared to 48% in those with moderate steatosis; $p = 0.04$) ; the GG genotype was significantly more frequent in subjects with moderate steatosis (28% compared to 5.26% in those with severe steatosis; $p = 0.03$); the frequency of the CG genotype was not significantly different among the groups ($p > 0.05$).

As the frequency of the genotypes was the same in the group of subjects without steatosis and the group of subjects with mild steatosis, we combined the two groups and compared them with the groups of subjects with moderate and severe steatosis.

This method of comparison showed that the frequency of the CC genotype was significantly higher in the group of subjects with severe steatosis (73.68% compared to 48% in moderate steatosis; $p = 0.04$); the frequency of the CG genotype was significantly higher in subjects with no or mild steatosis (50% compared to 24% in subjects with moderate steatosis; $p = 0.03$ and 21.05% in subjects with severe steatosis; $p = 0.03$); the frequency of the GG genotype was significantly higher in subjects with moderate steatosis (28% versus 0% in subjects with normal liver or mild steatosis; $p = 0.004$).

Furthermore, we divided the group of subjects into two groups: those with no or mild steatosis and those with moderate or severe steatosis.

The frequency of the GG genotype was significantly higher in the group of subjects with moderate or severe steatosis (18.18% in comparison with 0% in the group of subjects with no or mild steatosis; $p = 0.01$).

No significant differences were found in terms of the PNPLA3 genotype regarding the degree of subclinical atherosclerosis, the presence or the components of the metabolic syndrome, or HOMA-IR index.

The frequency of the GG genotype was significantly higher in the group of subjects with moderate or severe steatosis (18.18% in comparison with 0% in the group of subjects with no or mild steatosis; $p = 0.01$).

Discussions

In this group of patients with diabetes, the prevalence of hepatic steatosis was very high (approximately 90%), and these results were similar to other recent data (a percentage varying from 50 to 90%) (Mantovani et al, 2017; Hazlehurst et al, 2016; Atan et al, 2017; Estes et al, 2018). The fact that the prevalence of steatosis obtained in our research is at the upper limit of the results of other studies could be explained by the food habits specific to our region (high saturated fats) aggravated by a sedentary lifestyle (especially for those in the urban environment).

The GG genotype was associated with an increase in the hepatic fat content. In this population of subjects with diabetes, the results confirmed the influence of the PNPLA3 polymorphism on the hepatic triglyceride content. An important finding of our research was the lack of statistically significant associations between the PNPLA3 genotypes and the components of metabolic syndrome. In the subgroups resulting from PNPLA3 genotyping, the comparison of the average CIMT values indicated a statistically significant difference between the CC and CG genotypes ($p = 0.01$). The lack of associations with the components of the metabolic syndrome suggested that the presence of the G allele was not connected with metabolic disorders in subjects with type 2 diabetes mellitus. These data were in accord with other studies, which proved that in the general population, the PNPLA3 polymorphism was closely connected with the hepatic fatty content, independent of adiposity or insulin resistance. A study that included 330 subjects and used magnetic resonance spectroscopy as a diagnostic method proved that carriers of the G allele presented a significantly higher hepatic fat content compared to individuals homozygous for the C allele (Kantartzis et al, 2009). This study suggested that adiponutrin could be a key factor in understanding the mechanisms involved in the differentiation of benign fatty liver and fatty liver with metabolic consequences. Nonetheless, the PNPLA3 gene could manifest itself better in the company of certain predisposing factors of hepatic injury (obesity, alcohol consumption, hepatic viruses) (Trepo et al, 2016).

PNPLA3 does not affect the components of metabolic syndrome (Speliotes et al, 2010). In our study, the prevalence of metabolic syndrome did not present differences regarding the PNPLA3 genotypes, which indicated that the management of metabolic factors was important regardless of genotype.

We can consider the PNPLA3 genotype to be an important predictor of the degree of hepatic fat loading, even in subjects without metabolic syndrome. Hepatic steatosis can be viewed as a new component or even as a cause of this syndrome (Kim et al, 2018). Nonetheless, the reason for which certain subjects without a metabolic risk develop hepatic steatosis remains incompletely clarified.

Although our study did not prove positive correlations between the presence of the GG genotype and subclinical atherosclerosis (increased CIMT), other authors reported contradictory results but only in subjects younger than 50 years of age (Petta et al, 2013). A possible explanation is that in young subjects who are not yet exposed to increased age-related cardiovascular risk, PNPLA3 can manifest its atherogenic role better. Moreover, the different types of fat distribution may impact on the relationship of the genotypes with hepatic steatosis and CIMT (Stefan, 2020). This theory justifies the lack of a direct correlation between the PNPLA3 polymorphism and subclinical atherosclerosis in our study,

as our study group consisted mainly of elderly people with a variety of associated cardiovascular risk factors (type 2 diabetes mellitus, obesity, dyslipidemia).One of the major limitations of our study is the limited number of subjects and the lack of the healthy control group.

Conclusions

Although the GG genotype presented a higher frequency in diabetic subjects with high hepatic fatty loading, it was not significantly correlated with subclinical atherosclerosis, insulin resistance (HOMA-IR), or elements of metabolic syndrome.

Our results suggest that PNPLA3 does not independently influence cardiovascular risk in patients with type 2 diabetes mellitus. The hypothesis that PNPLA3 may have a cardioprotective effect requires future confirmation.

I.2.2.2. Cardiovascular disease and diabetes mellitus

For a better approach of the cardiovascular system, I obtained the competence certificate in General Echocardiography, by national contest-examination, in 2006, at "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, coordinated by Prof. Radu CAMPEANU, head of Heart Institute, Competence in General Echocardigraphy (Certificate series 017683/29.11.2006), provided by Health Ministry, for Postgraduates doctors.

I.2.2.2.1. The role played by novel inflammatory markers in assessment of peripheral artery disease

Introduction

Atherosclerosis is a multifactorial process in which inflammatory markers have both therapeutic and prognostic roles. Recent studies bring into question the importance of assessing new inflammatory markers in relation to the severity of peripheral artery disease (PAD), such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-C-reactive protein ratio (LCR) (Björkegren et al, 2022).

Materials and Methods

We conducted a retrospective and descriptive study including 652 patients with PAD, who were divided into two groups according to the severity of the ankle–brachial index value: mild and moderate obstruction (257 patients) and severe obstruction (395 patients). We evaluated demographics, anthropometric data and clinical and paraclinical parameters in relation to the novel inflammatory biomarkers mentioned above.

Results

Weight ($p = 0.048$), smoking ($p = 0.033$), the number of cardiovascular risk factors ($p = 0.041$), NLR ($p = 0.037$), LCR ($p = 0.041$) and PLR ($p = 0.019$), the presence of gangrene ($p = 0.001$) and the number of lesions detected via peripheral angiography ($p < 0.001$) were statistically significant parameters in our study. For the group of patients with severe obstruction, all three inflammatory biomarkers were statistically significantly correlated with a serum low-density lipoprotein–cholesterol level, the number of cardiovascular risk factors, rest pain, gangrene and a risk of amputation. In addition, directly proportional relationships were found between NLR, PLR and the number of stenotic lesions ($p = 0.018$, $p = 0.016$). Also, NLR (area under the curve = 0.682, $p = 0.010$) and PLR (AUC = 0.692, $p = 0.006$) were predictors associated with a high risk of amputation in patients with an ABI < 0.5.

Discussions

Atherosclerosis is a multifactorial process in which inflammatory status plays a role in determining both the appearance of lesions and their progression and, therefore, in increasing the risk of an acute cardiovascular event (Alexander et al, 2021; Berenji et al,

2020). In total, 84.7% of the patients enrolled in our study were males, with a mean age of 66.46 ± 10.47 years. By analyzing a broad spectrum of parameters, we highlighted the role played by the proposed inflammatory biomarkers (NLR, PLR, LCR) in the management of PAD and their progVarious degrees of inflammation have been identified at all stages of PAD, with this association also being thoroughly researched. Some studies indicate a stronger association with PAD than with coronary artery disease, suggesting the presence of different predominant substrates (Tunstall-Pedoe et al, 2017). In our study, serum hs-CRP levels were elevated, regardless of the severity of obstruction, representing a statistically significant inflammatory marker ($p = 0.023$). Also, regarding medium- and long-term evolution of hs-CRP, it has been shown that in patients with PAD, high levels at the time of the first revascularization intervention and their persistence at 3.6 years of follow-up are associated with an independent increase in all-cause, cardiovascular and malignancy-related mortality, with these results being supported by other similar research (Saenz-Pipaon et al, 2021; Fukase et al 2021). The mean serum values of NLR ($p = 0.037$), LCR ($p = 0.041$) and PLR ($p = 0.019$) were higher in patients with severe obstruction, as well as statistically significant biomarkers in our analyzed group. Similar results have been reported by other investigators in the literature, with the calculation of these biological parameters having both therapeutic and prognostic valuenostic implications. Similar to the NLR, the PLR has a predictive value regarding the risk of an acute vascular event; in the case of patients with PAD, the existence of a value of more than 150 is associated with a relative risk about two times higher than that of critical atherosclerotic lesions (Gary et al, 2013). Liu et al. (Liu et al, 2019) analyzed a cohort of 355 diabetic patients, in whom they assessed the risk of developing PAD and identified NLR and PLR as predictors associated with the development and progression of atherosclerotic processes in this category of patients, finding evidence of the superiority of PLR. The validity of PLR's use as an inflammatory marker is secondary to the proinflammatory effect exerted by platelets (Walzik et al, 2021). Initially investigated in various oncological clinical trials (Stojkovic Lalosevic et al, 2019), this biomarker has increasingly broad validity as a predictor of moderate-to-severe functional decline in PAD patients, as demonstrated above. PLR is another biomarker that plays a prognostic role in the management of patients with PAD, with elevated titers being associated with a high risk of critical ischemia or acute vascular events (odds ratio of 1.9 for PLR > 150) (Gary et al, 2013).

Our study presents several limitations due to the lack of follow-up. The heterogeneity of the study group or the potential risk associated with the inclusion of patients with elevated serum CRP values due to associated infections are additional aspects that may influence the obtained results. We excluded records in which medical data were unavailable. This step was taken to minimize the risk of misclassification, introducing a limited risk of selection bias. Our future research direction will be to investigate the influence of the proposed markers (NLR, PLR, CSF) on the predictive value of amputation risk in relation to a series of biochemical or clinical models, such as PREVENT III or the BASIL model, that exist in the literature (Mills, 2023).

Conclusions

In our study, we demonstrated the predictive value of the analyzed inflammatory biomarkers and the importance of their assessment in patients with severe obstruction and a high risk of amputation.

NLR and PLR are predictors used in patients with ABI values below 0.5 and a risk of amputation, thus making them parameters with both therapeutic and prognostic value. NLR, PLR and WMR are easy-to-determine and reproducible parameters, which can be easily used in daily practice, as they also have therapeutic and prognostic value among patients with PAD.

I.2.2.2.2. Advantages of thoracic electrical bioimpedance used for hypertension control in metabolic syndrome patients

Introduction

The metabolic syndrome is considered a public health problem, given its association with a high cardiovascular risk, as well as its high prevalence in the adult population (Alberti et al, 2005).

Hypertension is currently considered the most important risk factor for cardiovascular conditions, as the relation between hypertension and the risk of cardiovascular events have been continuous, constant, and independent of other risk factors.

Hypertension is a common component of the MS and also a factor that is highly susceptible to change (Cornelissen et al, 2005; Nguyen et al, 2008). Paradoxically, despite the impressive therapeutic options that are currently available, recent studies conducted continue to apply (meaning that only half of the people suffering from hypertension are aware of this diagnosis, and only half of them get treatment, and less than a quarter of the people receiving treatment attain the therapeutic targets) (Adair et al, 2005; ALLHAT Officers, 2002). Current hypertension approaches disregard the fact that hypertension patients may have different hemodynamic statuses, since their blood flow is not usually measured (Lakka et al, 2002). According to a new approach, in most cases HT has hemodynamic origin, and it should be treated as such. The hemodynamic HT treatment involves tackling hemodynamic modulators the deviations of which are identified by the HOTMAN system, which relies on the thoracic electrical bioimpedance method. According to literature data, hypertension therapy guided according to hemodynamic parameters determined by thoracic electrical bioimpedance increases the blood pressure control rate. Assessing the plasma volume without hemodynamic methods is often difficult (Adair et al, 2005; Nguyen et al, 2008). The TEB method identifies the hemodynamic disorders (causes) associated with hypertension (effect) and enables the doctor to choose the best anti-hypertension therapy for each patient, allowing blood pressure and perfusion to reach normal levels (Raiijmakers et al, 1999).

Material and methods

The study included 30 patients suffering from metabolic syndrome and uncontrolled hypertension, who declared treatment compliance. Hypertension was considered uncontrolled when the blood pressure was 140/90 mmHg or above, in patients that did not suffer from diabetes mellitus, and 130/80 mmHg or above, in patients suffering from DM. The patients were monitored for 6 months, during which 4 sets of hemodynamic measurements were performed, namely on their inclusion in the study and after 1, 3 and 6 months, respectively.

Vasoactivity, blood volume, inotropy and chronotropy were determined. The values were generated automatically by a HOTMAN system after h (demographic and anthropometric data, blood pressure, temperature, haemoglobin values). Treatment modulation was done depending on the hemodynamic profile of each patient. The interpretation of the results provided by TEB relied on normal percentage for blood volume and inotropy. The data were computer processed using the SPSS program.

Results

Blood pressure (BP) was initially uncontrolled in all the patients, and after a month the percentage of patients with uncontrolled blood pressure dropped to about 45% and then to 20% after 6 months 2=73.51; GL=3; p<0,001) (Figure 2.2).

The cases under survey exhibited significant hypervolemia only at the beginning of the study (53.3%), as later monitoring only revealed some cases of mild hypervolemia ranging from 64% after one 2=33.17; GL=4; p=0.000003) (Figure 2.3).

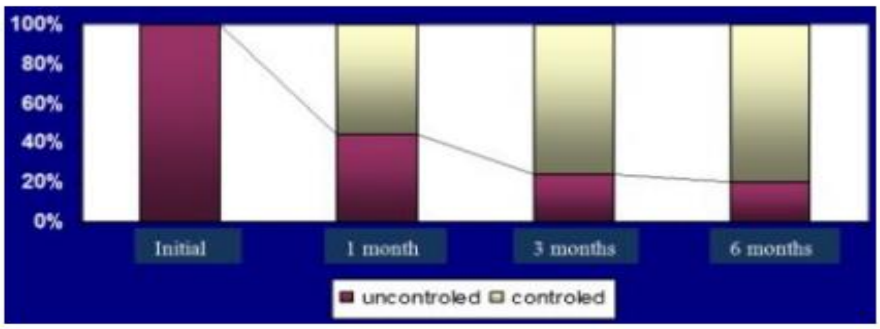

Fig. 2.2. Patient distribution depending on blood pressure

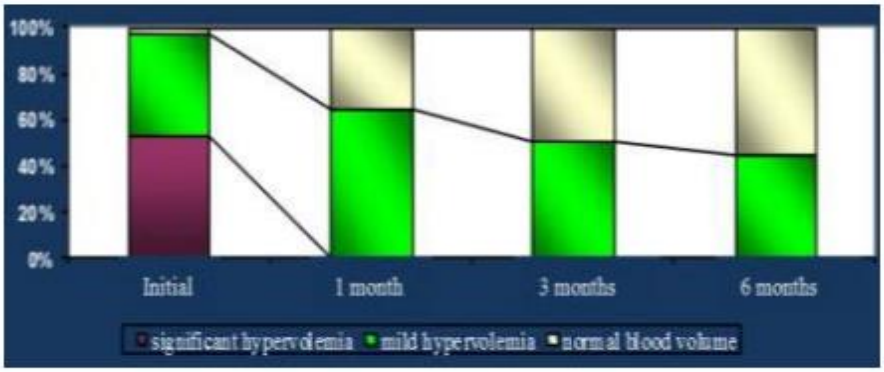

Fig. 2.3. Patient distribution depending on blood volume

One should note that if initially 98% of the patients associated uncontrolled BP with altered blood volume, after one month 60% of the patients with altered blood volume experienced high BP, the relative risk in these patients being about 2 times higher (RR=1.98). After 3 months, about 40% of the patients associated uncontrolled BP with altered blood volume values, in whom the relative risk was over 2 times higher (RR=2.38). At the end of the study, uncontrolled BP was associated with altered blood volume levels in about 30% of the patients, who still had a risk of about 1.71 times higher, yet the frequency distribution does not reveal statistically significant differences (p=0.261) able to support this risk ratio (Figure 2.4).

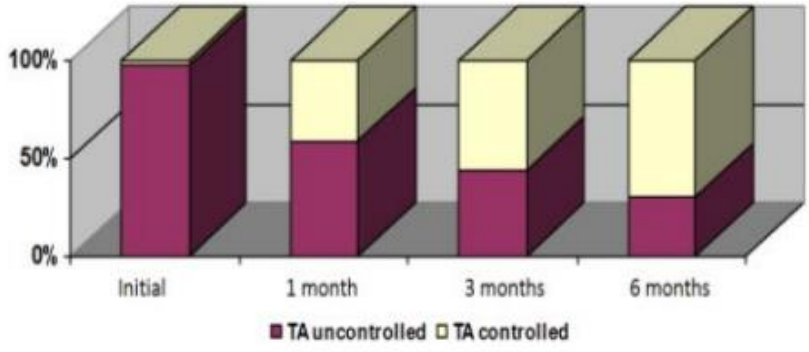

Fig. 2.4. Distribution of cases of altered blood volume depending on blood pressure

In the cases we studied, we found significant vasoconstriction only at the beginning of the study (33.3%). The following monitoring processed revealed only cases of mild vasoconstriction ranging from 58% after one month to 36% after 6 months 2=15.41; GL=4; p=0.001) (Figure 2.5).

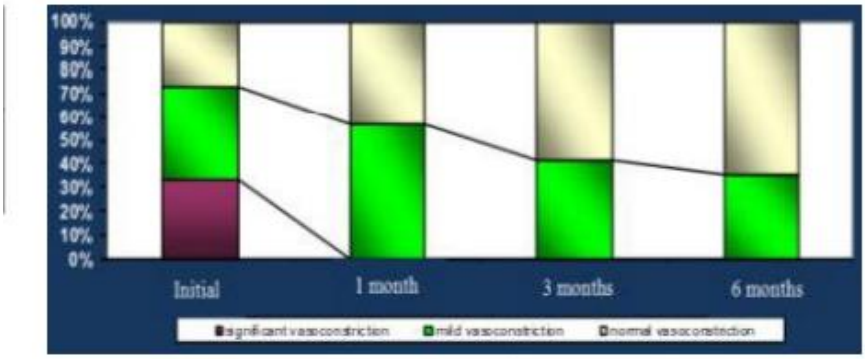

Fig. 2.5. Patient distribution depending on vasoactivity

At first, 73% of the patients associated uncontrolled BP with altered vasoactivity, and after one month, 54% of the patients with altered vasoactivity suffered from uncontrolled blood pressure, the relative risk in these patients being 1.46 times higher, yet the frequency distribution does not reveal statistically significant differences (p=0.238) able to support this risk ratio. After 3 months, we noticed a relation between uncontrolled blood pressure and altered vasoactivity in less than 20% of the patients, in whom the relative risk was 1.43 times higher, yet the frequency distribution does not reveal statistically significant differences (p=0.548) able to support this risk ratio. At the end of the study, uncontrolled BP was associated with altered vasoactivity in about 50% of these patients, who experienced a relative risk 2.4 times higher in the case of such an association.

Ninety-five percent of the patients initially associated blood volume with altered vasoactivity, whereas after only one month 69% of the patients with altered vasoactivity suffered from mild blood volume changes, the relative risk in these patients being 1.24 times higher, yet the frequency distribution does not reveal statistically significant differences (p=0.639)able to support this risk ratio. After 3 months, we noticed an association of hypervolemia with altered vasoactivity in over 60% of the patients, with a relative risk 1.64 times higher, yet the frequency distribution does not reveal statistically significant differences (p=0.280) able to support this risk ratio. At the end of the study, hypervolemia was associated with altered vasoactivity in 69% of the patients, and we detected a relative risk 2.75 times higher in the case of such an association (Figure 2.6).

We noticed that the hyperdynamic status dropped from 20% to 7% in all the moments where it was analysed. The hypodynamic status also decreased from an initial percentage of 29% to 7% after 6 months of monitoring. The frequency distribution had 2 =17.13; GL=4; p=0.0007) (Figure 2.7).

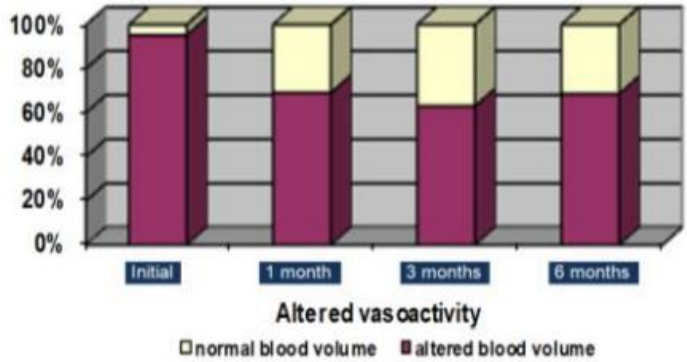

Fig. 2.6. Distribution of altered vasoactivity cases depending on blood volume

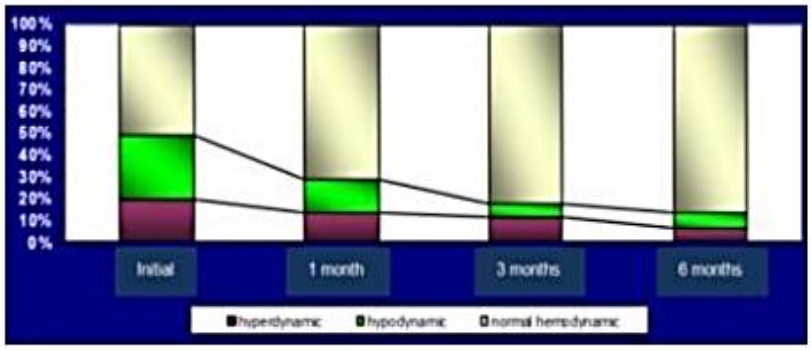

Fig. 2.7. Patient distribution depending on hemodynamic status

At first, all the patients associate uncontrolled blood pressure with altered hemodynamic status, which was also revealed at the evaluations conducted after one and even 3 months, despite the lower number of people with uncontrolled blood pressure, yet we were unable to identify any relative risk of this association. At the end of the study, uncontrolled blood pressure was associated with altered hemodynamic status in few over 60% of the patients, the relative risk being 8.44 times higher in the case of this association (Table 2.III).

Table 2.III. Estimation of risk parameters in blood pressure-hemodynamic status association

Parameter	Estimated value	95% CI	
		min	max
6 months			
Opportunity ratio (OR)	14.40	1.60	11.70
Risk ratio (RR)	8.44	1.82	39.16
Chi-square (χ^2)	6.82		
Significance level	**p=0.009**		

Initially, 98% of the patients associated blood volume with altered hemodynamic status, and after one month 92% of the patients with altered hemodynamic status experienced mild blood volume changes, the relative risk in these patients being 6.62 times higher.

After 3 months, we noted an association between hemodynamic status and altered blood volume in over 60% of the patients, with a relative risk 6.7 times higher. At the end of the study, altered hemodynamic status was associated with altered blood volume in 69% of the patients, with a relative risk 6.25 times higher in the case of this association.

Initially, 73% of the patients associated altered vasoactivity with altered hemodynamic status, and after one month 50% of the patients with altered hemodynamic status experienced mild vasoactivity changes, the relative risk in these patients being 6.62 times higher. After 3 months, an association between hemodynamic status and altered vasoactivity was reported in fewer than 40% of the patients, with a relative risk 6.7 times higher. At the end of the study, altered hemodynamic status was associated with altered vasoactivity in 50% of the patients, with a relative risk 6.25 times higher in the case of this association.

Discussions

Current hypertension approaches disregard the fact that hypertension patients may have different hemodynamic statuses, since their blood flow is not usually measured (Lakka et al, 2002). According to a new approach, in most cases HT has hemodynamic origin, and it should be treated as such.

Hypertension is associated with hemodynamic modulator disorders, and it should therefore be treated as a hemodynamic origin condition and not as a mere rise in the blood pressure values. Thoracic electrical bioimpedance is a simple, rapid and non-invasive method, which is useful for practitioners when examining hypertension patients and which identifies hemodynamic deviations associated with hypertension. A hemodynamic approach of hypertension patients enables the doctor to choose the best specific therapy for each patient.

According to our study results, hemodynamically guided anti-hypertension therapy improves the hypertension control rate. In our study, normal blood pressure was achieved, accompanied by normal dynamic status, i.e., normal tissue perfusion, which obviously led to better quality of life.

Conclusions

Hypertension is associated with hemodynamic modulator disorders, and it should therefore be treated as a hemodynamic origin condition and not as a mere rise in the blood pressure values. Thoracic electrical bioimpedance is a simple, rapid and non-invasive method, which is useful for practitioners when examining hypertension patients and which identifies hemodynamic deviations associated with hypertension.

Bioelectrical impedance analysis is a technique widely used for estimating body composition and health-related parameters. The technology is relatively simple, quick, and non-invasive, and is currently used globally . The parameters provided can be used to estimate body composition (fat, fat-free mass, total-body water and its compartments). Moreover, raw measurements including resistance, and impedance vector length can also be used to track health-related markers, including hydration and disease-prognostic, and general health status. Body composition shows profound variability in association with age, sex, race and ethnicity, geographic ancestry, lifestyle, and health status.

A hemodynamic approach of hypertension patients enables the doctor to choose the best specific therapy for each patient. According to our study results, hemodynamically guided anti-hypertension therapy improves the hypertension control rate. In our study, normal blood pressure was achieved, accompanied by normal dynamic status, i.e., normal tissue perfusion, which obviously led to better quality of life.

I.2.2.2.3. Cardiovascular risk amelioration in the metabolic syndrome – primary prophylaxis vs. Secondary prophylaxis

Introduction

Even though the end of the XX-th century and the beginning of the XXI-st century are marked by an avalanche of studies, trials and guides, by an unprecedented development of the medical technique, the results are not equally hopeful when we talk about the prophylaxis of these disorders. This context explains why the need for effective prevention strategies of the cardiovascular disorders has become all over the world an emergency which can be no longer adjourned (Assmann et al, 2005; Van Horn et al, 2008).

The main purpose of the cardiovascular risk assessment in the metabolic syndrome (MetS) is to identify the persons who need pharmacological treatment. The answer to the question of whether the cardiovascular risk associated to the metabolic syndrome is higher than the sum of the risks of its components seems to be affirmative.

Epidemiological studies suggest that the association of the risk factors lead to the increase of the risk more than the sum of the risks which accompany each factor taken individually. The cardiovascular risk seems to increase in a geometrical manner, not in a linear one, this phenomenon being called multiplicative risk (Gami et al, 2007).

In practice, the cardiovascular risk quantification is carried out in a different manner. The Framingham and SCORE risk charts are used for the general population and for the patients with type 1 DM and the UKPDS soft is used for type 2 DM. DESIR chart was suggested for the quantification of the cardiovascular risk in the metabolic syndrome, but it has not been validated yet. The clinical studies confirmed the excessive cardiovascular risk in persons with MetS syndrome, the excessive risk being more accentuated in women, but also in those with previous diagnosis of type 2 diabetes mellitus, the metabolic syndrome being associated to an increase of the cardiovascular morbidity and death rate (Rutter et al, 2006).

This study aims at assessing the control of the cardiovascular risk factors in patients with MetS with no cardiovascular events vs. Patients with MetS and cardiovascular disorders.

Material and methods

The study was carried out over the period from January to December 2012, in the Medical Clinic no. V of the "Căi Ferate" Hospital of Iași and included a number of 107 patients, who were distributed in two groups. The first group included patients with MetS with no cardiovascular disorder and the second one included patients with MetS and cardiovascular disorder.

The inclusion criteria in the study were represented by: patients diagnosed with MetS according to the definition with/without cardiovascular disorder, male persons aged more than 45 years and less than 80 years and women at post-menopause and aged less than 80 years.

The exclusion criteria from the study were represented by patients' refusal; men aged more than 80 years and less than 45 years, patients in pre-menopause, previous physical troubles, patients under hormonal treatment.

The investigation methods were represented by the anamnesis, the clinical examination and the para-clinical investigations, which included: EKG, glycaemia, lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) and creatinine.

The cardiovascular disorder was represented by the ischemic heart disease (heart failure, angina pectoris, revascularization, heart block), cerebral vascular accidents (ischemic, hemorrhagic, transitory), obliterative arteriopathy of the lower members, chronic kidney disease, advanced retinopathy.

Results

Patients' distribution in the two groups was performed according to the presence or absence of the cardiovascular disorder. Therefore, group no. 1 (69 cases) included the patients with MetS with no cardiovascular event and group no. 2 (38 cases) included the patients with MetS and cardiovascular disorders.

The results have shown that only 51 of the 107 patients were informed with regard to the risk factors that they presented, but most of them (86.8%) had cardiovascular disorders. All the patients who presented cardiovascular events received drug treatment and only a percentage of 68.1% of those with no cardiovascular disorder received this recommendation.

Fewer than a half of the patients in the first group were compliant to the recommended treatment, but their percentage increases (78.9%) in the ones with cardiovascular disorders. The analysis of these data shows that the appearance of the manifest clinical cardiovascular disorder determines the increase of the compliance to treatment.

According to the data from literature, the high blood pressure is a frequent individual component of the metabolic syndrome, different studies reporting it as being present in 50-76% of the cases. Within the two groups, the prevalence of the high blood pressure (HBP) was almost equal, 57% in the first group, respectively 55% in the second.

Even though the prevalence of the HBP in the studied groups does not present significant differences (p>0.05), when the control of this risk factor was analysed, we noticed that in the patients with MetS and cardiovascular disorder, the HBP was controlled therapeutically in a proportion almost double as compared to the group without manifest cardiovascular disorder.

DM was previously diagnosed in 14% of the studied patients, 12% in the first group and 18.4% in the second. However, we must notice that these percentages show an increased prevalence of DM in the studied groups as compared to the prevalence in the general population, where there is a frequency of 5%, according to certain studies carried out in Romania.

The analysis of the glycaemia values did not show significant differences (p>0.05) between the two groups, but the severe desequilibrated DM was more frequent among the patients of the first group.

Glycaemia increase is frequently associated to the atherosclerotic vascular disease. Even beyond the glycaemia values which define diabetes mellitus, there is a close connection between glycaemia and the cardiovascular risk.

The exact mechanism by means of which the hyperglycaemia increases the cardiovascular risk is not fully elucidated.

One of the mechanisms seems to be the glycosylation of the circulating proteins, which determine the increase of aterogenity of the LDL-cholesterol and, subsequently, accelerates the atherosclerosis.

The highest prevalence of atherosclerosis registers in the situation of the simultaneous presence of diabetes mellitus and the metabolic syndrome, but one must consider that the cardiovascular risk in persons with metabolic syndrome without diabetes shall be treated with the same intensity as in the case of those with diabetes without metabolic syndrome.

One can notice the increased prevalence (71.1%, respectively 57.9%) of the hypercholesterolemia among the studied subjects with metabolic syndrome (p=0.05) as compared to the general population, where according to the data in the literature this lipid abnormality is met with a frequency of almost 25%

We must highlight that the percentage of the patients with values of the total cholesterol below the risk level is higher in the second group, but at the same time we can notice that in more than a half of these patients the therapeutic goal was not achieved, even in the presence of the cardiovascular disorder.

The total cholesterol is considered as a strong risk predictor for the coronary disease. According to the Framingham, MRFIT and ARIC studies, the cardiovascular risk increases by 2-3% for each increase percentage of the concentration of the total cholesterol.

Initially, most of the studies documented a close connection between the level of the total cholesterol and the ischemic heart disease, but subsequently we noticed that in fact the LDL cholesterol is the dominant causal agent with atherogenic properties, correlated to the cardiovascular risk increase.

The results of multiple clinical studies (WOSCOPS, AFCAPS/Tex CAPS, ASCOTLLA, 4S, LIPID, HPS) proved that the decrease of the serum values of the LDL cholesterol is correlated to the cardiovascular risk decrease.

On the studied cases, we can notice that the number of subjects with increased LDL cholesterol is higher than the one of the patients who presented normal values of this parameter. The percentage is higher (65.2%) within the first group, as compared to the group with cardiovascular trouble (60.5%), (p>0.05) .

There are studies which proved that in the case of the asymptomatic persons with high risk of developing the atherosclerotic vascular disorder, with values of the total cholesterol below 190 mg/dl and the LDL-cholesterol below 115 mg/dl, the additional

reduction of the levels of these parameters to the target values recommended to the persons with manifest cardiovascular disorder is accompanied by a decrease of the cardiovascular risk.

These results show an increased prevalence of this lipid abnormality in the ratio normal/low HDL being of almost 2/1, ($p<0.05$) studied group, as compared to the general population, where according to the data provided by different studies, it appears with a frequency of 10-15%.

The guides indicate that a level of the HDL-cholesterol < 40 mg/dl represents a marker of an increased cardiovascular risk which must attract the attention of the clinician in the management of the LDL cholesterol and tensional values level.

Expectedly, the subjects with metabolic syndrome associate more frequently hypertriglyceridemia, its prevalence within this study being double as compared to the one mentioned in studies regarding the general population. Another remark is that in patients who presented cardiovascular disorder, the hypertriglyceridemia weight was higher as compared to those without this disorder.

The relationship between triglycerides and atherosclerosis continues to be a controversial topic, the prognostic independent role of triglycerides increase as compared to the other lipid fractions not being completely established. According to certain studies, hypertriglyceridemia was associated to an increased risk of atherosclerotic disease, but this association seems to be less strong than in the case of hypercholesterolemia.

Moreover, there is an obvious reverse correlation between the HDL-cholesterol and the triglycerides.

In the studied groups, there were no significant differences from a statistical point of view ($p>0.05$) with regard to triglycerides levels, but only an increased frequency (47.4%) of hypertriglyceridemia among the patients with cardiovascular disorder.

The data derived from the PROCAM study in patients with levels of the triglycerides show that hypertriglyceridemia was identified higher than 200 mg/dl.

The high weight of the obese subjects is also justified by the fact that obesity was defined by means of the waist circumference and not by the BMI.

We must also highlight the fact that even though most of the obese persons (90%) received the recommendation to lose weight, an insignificant percentage only (4%) has benefited by the guidance of a dietician and none of the groups included subjects who received medical treatment for obesity.

Discussions

There are several genetic and environmental factors which contribute to the development of both metabolic disorders which are summarized as metabolic syndrome and cardiovascular disease. It is conceivable that metabolic and cardiovascular disorders develop in parallel and can influence each other. Therefore, the term metabolic-vascular syndrome might be the most comprehensive description of this cluster of disease (Hanefeld et al, 2016).

According to the data from literature, the high blood pressure is a frequent individual component of the metabolic syndrome, different studies reporting it as being present in 50-76% of the cases (Hanefeld et al, 2016).

We must also highlight the fact that even though most of the obese persons (90%) received the recommendation to lose weight, an insignificant percentage only (4%) has benefited by the guidance of a dietician and none of the groups included subjects who received medical treatment for obesity.

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Glycaemia increase is frequently associated to the atherosclerotic vascular disease. Even beyond the glycaemia values which define diabetes mellitus, there is a close connection between glycaemia and the cardiovascular risk (Hanefeld et al, 2016).

The exact mechanism by means of which the hyperglycaemia increases the cardiovascular risk is not fully elucidated.

One of the mechanisms seems to be the glycosylation of the circulating proteins, which determine the increase of aterogenity of the LDL-cholesterol and, subsequently, accelerates the atherosclerosis.

The highest prevalence of atherosclerosis registers in the situation of the simultaneous presence of diabetes mellitus and the metabolic syndrome, but one must consider that the cardiovascular risk in persons with metabolic syndrome without diabetes shall be treated with the same intensity as in the case of those with diabetes without metabolic syndrome (Hanefeld et al, 2016).

Conclusions

The metabolic syndrome is an aggregation of risk factors which increases in a marked manner the cardiovascular risk.

The assessment of the total cardiovascular risk is an essential tool for guiding patients' management, because it reflects the combined effects of several risk factors which can interact, amplifying each other at times.

Cardiovascular risk decrease is the constant concern of the medical staff and it includes both the improvement of the lifestyle, which influences in a favourable manner all the risk factors, and the customized drug therapy.

In the studied groups, there were no significant statistical differences with regard to the values of the blood pressure, glycaemia, LDL-cholesterol and triglycerides. As concerns the levels of the total cholesterol and the HDL-cholesterol, the results of the study have shown significant differences between the group without cardiovascular disorder and the one with cardiovascular disorder.

The presence of the cardiovascular disorder per se frames the patient with MetS in the group of very high risk. The cardiovascular prevention supposes assisting the persons with absolutely reduced risk in maintaining this condition for the entire life and helping the persons with totally increased risk of cardiovascular disorder to reduce it.

I.2.2.2.4. Haematological characteristics in coronary patients with comorbidities

Introduction

Coronary artery disease (CAD) is a significant public health problem, with a substantial contribution to global morbidity and mortality, especially in low- and middle-income countries (Nowbar et al, 2014; Ralapanawa et al, 2021). Current guidelines firmly recommend enrollment in a comprehensive cardiovascular rehabilitation (CR) program after CAD diagnosis or revascularization, with proven beneficial effects on cardiovascular and all-cause mortality and individual quality of life (QoL) (Goel et al, 2011; Ambrosetti et al, 2020; Price et al, 2016; Thomas et al, 2019). CAD is associated with a significant impact on the individual's exercise.

Functional capacity (FC) is a strong, independent prognostic factor in heart failure (HF) (Lala et al, 2021) and CAD (Keteyian et al, 2008). The prognostic value of FC is independent and additive to other well established mortality predictors such as left ventricular ejection fraction (LVEF), smoking, hypertension (HTN), dyslipidemia and

diabetes (Mezzani et al, 2013; Guazzi et al, 2012; Forman et al, 2017). Peak oxygen uptake (VO2 max) assessed via cardiopulmonary exercise testing (CPET) is an objective measure of FC, and an independent predictor of cardiovascular morbidity and mortality in patients with CAD (Mezzani et al, 2013; Guazzi et al, 2012; Hung et al, 2014; Mikkelsen et al, 2020).

However, CPET availability remains limited, especially in developing countries. Systemic inflammation plays a major role in CAD etiopathogenesis (Yoshikane et al, 2007) and routine inflammatory biomarkers (complete blood count, C-reactive protein) have proven their role for both acute and long-term cardiovascular risk assessment (Erikssen et al 2000; Andresdottir et al, 2003; Strang et al, 2014).

Physical activity decreases systemic markers of inflammation, thrombosis and endothelial dysfunction, and has a key role in preventing CAD (Colbert et al, 2004; Koenig et al, 2000; Linke et al, 2001).

The platelet to lymphocyte ratio (PLR) is an integrated reflection of two important opposite inflammatory pathways that can be easily calculated from a complete blood count. PLR initially served as a prognostic biomarker in neoplastic diseases (Krenn-Pilko et al, 2014; You et al, 2016), but has recently been studied in HF (Durmus et al, 2015; Heidarpour et al, 2021; Ye et al, 2019), ACS (Sun et al, 2017; Dong et al, 2021; Azab et al, 2012; Ugur et al, 2014; Willim et al, 2021), atrial fibrillation (Zuo et al, 2020; Dereli et al, 2019), deep venous thrombosis (Velioglu et al, 2019), PCI (Zhen et al, 2020; Karatas et al, 2016; Ayça et al, 2015) and infective endocarditis (Meshaal et al, 2019).

The neutrophil to lymphocyte ratio (NLR) is another readily available biomarker of inflammation in cardiac and non-cardiovascular disorders (Tamhane et al, 2008; Park et al, 2013; Akpek et al, 2012). In previous reports, the NLR appeared to be a predictor of cardiovascular events and mortality in patients with stable CAD and was associated with coronary atherosclerosis severity (Papa et al, 2008; Kaya et al, 2014). NLR was also used as a predictor for functional capacity in patients undergoing CR (Okan et al, 2020) and a predictor of lipid-lowering effectiveness in patients with familial hypercholesterolemia and atherosclerotic cardiovascular disease (Scicali et al, 2021).

However, the current literature offers limited data regarding the role of these readily available inflammatory biomarkers in predicting exercise performance in CAD patients. We therefore hypothesized that impaired cardiovascular performance (as defined by CPET) could be predicted by NLR and PLR in individuals with stable CAD and recent elective PCI. The aim of this study was to evaluate the utility of two readily available inflammatory biomarkers (NLR and PLR) in predicting poor FC in patients with CAD and recent elective PCI.

Materials and Methods

We conducted a retrospective cross-sectional study of all patients with stable CAD and recent elective PCI, referred for phase II CR in the Cardiovascular Unit of the Clinical Rehabilitation Hospital in Ias, i over a period of 18 months (January 2020–June 2021). Inclusion criteria were as follows: elective PCI performed for stable CAD during the previous 3 months and CPET performed upon admission (Figure 2.8).

Patients with ACSduring the previous 12 months, anemia (hemoglobin <12 g/dL in females and <13 g/dL in males), atrial fibrillation, moderate or severe valvular heart disease, decompensated congestive heart failure, any congenital heart disease or any other severe chronic disease except CAD were excluded from this analysis. All patients had a negative COVID-19 PCR upon admission.

Socio-demographic, clinical, biological, CPET and echocardiographic data were extracted from hospital medical records.

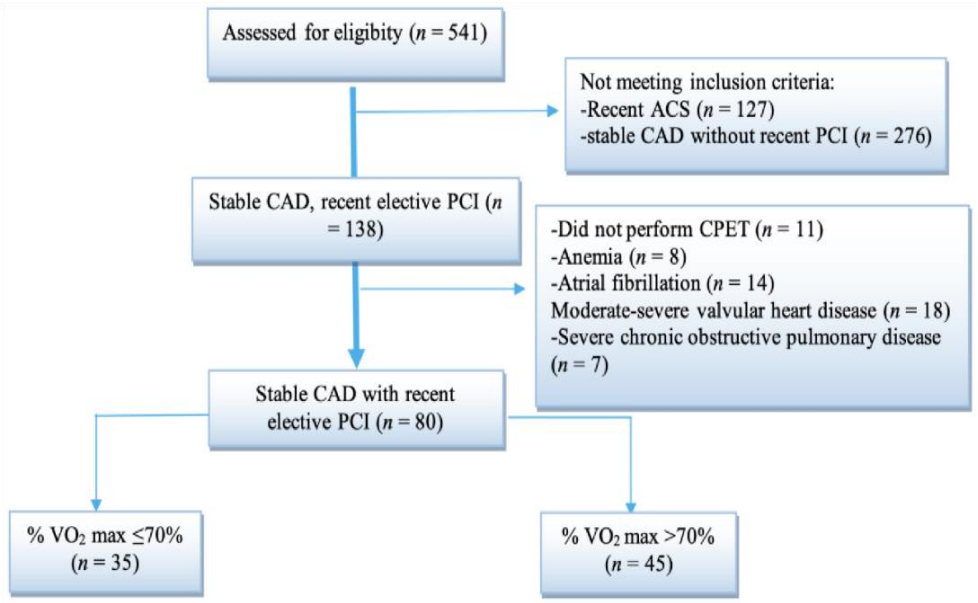


Fig. 2.8. Flow chart diagram of patients hospitalized in the Cardiovascular Rehabilitation Clinic Unit between January 2020 and June 2021, CAD—coronary artery disease, PCI-percutaneous coronary intervention, ACS—acute coronary syndrome, CPET-cardiopulmonary exercise test, % VO2 max—percentage of the predicted value of maximal oxygen uptake.

All patients were under optimal CAD treatment, according to current guidelines (Knuuti et al, 2020). Obesity was defined as a body mass index (BMI) ≥30 kg/m2. High blood pressure (HBP) was defined as current BP lowering treatment, prior diagnosis of HBP, resting systolic blood pressure (SBP) greater than 140 or resting diastolic blood pressure (DBP) greater than 90 mmHg (Williams et al, 2018). Diabetes was defined as current antidiabetic treatment, previous diabetes diagnosis, fasting glucose ≥126 mg/dL on two separate occasions or a value for glycosylated hemoglobin ≥6.5% (American Diabetes Association, 2019; WHO, 2006; WHO, 2011).

According to hospital protocol, blood samples were collected a jeun, in the morning upon admission, by qualified medical professionals. All blood samples were processed in the hospital's laboratory. Complete blood count was processed using the Pentra DF Nexus Hematology System® (Horiba Healthcare, Kyoto, Japan). Biochemistry was processed using the Transasia XL 1000 Fully Automated Biochemistry Analyzer (Transasia Bio-Medicals Ltd., Mumbai, India). We recorded the following parameters: platelet count, neutrophil count, lymphocyte count, C-reactive protein (CRP), low-density lipoprotein (LDL) and glycated hemoglobin (HbA1c). NLR was calculated using the absolute neutrophil (N) and lymphocyte (L) values from the complete blood count, using the formula: NLR = N/L. PLR was calculated using the absolute platelets (P) and lymphocyte (L) values from the complete blood count, using the formula: PLR = P/L.

Standardized transthoracic echocardiography (2D, Doppler) was performed by experienced sonographists according to current EACVI guidelines (Lancellotti et al, 2017) (Toshiba Aplio 500 Series, Toshiba Medical Systems Corporation, Otawara, Tochigi, Japan) prior to CPET evaluation. LVEF was calculated using Simpson biplane method.

CPET was performed by a certified pulmonologist on the Piston PRE-201 ergospirometer (Piston Ltd., Budapest, Hungary). According to hospital protocol, CPET was performed in the morning of the second day of hospitalization, in order to establish functional capacity and target heart rate for exercise rehabilitation. Each patient signed a written informed consent before the test. The test consisted of a 2 min resting period followed by 3 min warm up at 0 W followed by standard incremental exercise protocol of 15 W/min.

The CPET was performed under continuous heart rate (HR), 12-lead ECG (electrocardiographic) and pulse oximetry (SpO2) monitoring. Blood pressure was recorded every 2 min. Indications for exercise termination included exhaustion, myocardial ischemia, complex ventricular arrythmia, grade 2 or 3 atrio-ventricular block, a sudden drop in BP levels > 20 mmHg, extreme BP elevation (SBP > 220 mmHg, DBP > 120 mmHg), SpO2 < 80%, confusion or severe dizziness. We recorded the following parameters: resting SBP and DBP (measured with a manual sphygmomanometer immediately prior to the CPET), resting HR (recorded on the resting ECG performed immediately prior to the CPET), % peak HR (maximum heart rate relative to predicted normal for age (220—age in years)), % peak WR (maximum workload relative to predicted normal according to age and sex, automatically calculated by the ergospirometer software) and % VO2 max (maximum oxygen uptake (highest value, mean of 20 s) relative to predicted normal according to age and sex, automatically calculated by the ergospirometer software). Functional capacity was assessed according % VO2 max, using a convention proposed by Cooper et al., as follows: >80%—normal, 71–80%— mildly reduced, 51–70%—moderately reduced and ≤50%—severely reduced [55]. Due to a relatively small number of enrolled patients, we divided our study group as follows: poor FC (% VO2 max ≤70) and preserved FC (% VO2 max >70).

Statistical Analysis

Data analysis was performed using SPSS 20.0 (Statistical Package for the Social Sciences, Chicago, IL, USA). For continuous data, the normality of distribution was assessed by Shapiro–Wilk test. Data are presented as mean ± standard deviation (SD) for continuous variables with normal distribution, or as median with interquartile range for non-normally distributed continuous variables. Categorical variables are presented as number of cases with percent frequency. An independent samples T-test was used to compare continuous variables with normal distribution. A non-parametric Mann–Whitney's U test was applied to compare the variables not satisfying the assumption of normality. Categorical comparisons were performed using Chi-square test or Fisher's exact test (when the expected number of values in any of the cells of a contingency table was ≤5). Variables with $p < 0.05$ in the univariate analysis were included in the multivariate logistic regression model, to assess the independent predictors of poor FC (% VO2 max ≤ 70). The results are presented as odds ratio (OR) with 95% confidence intervals (CIs). Receiver operating characteristic (ROC) curve analysis was done to determine the optimum cut-off value of PLR in predicting poor FC of CAD patients and recent PCI. Correlation analyses, calculating Pearson correlation coefficients, were assessed considering normally distributed and linearly related variables. A two-sided p value < 0.05 was considered significant for all analyses.

Results

Table 2.IV illustrates clinical and demographic features, laboratory findings and exercise measurements of the 80 analyzed patients (age range: 34–79 years old) and a univariate analysis of the two subgroups according to the values of % VO2 max. Age and the presence of cardiometabolic comorbidities (obesity, diabetes, HTN, LDL level) were similar among the two subgroups.

Our analysis included 35 patients with % VO2 ≤70 and 45 patients with % VO2 >70. Among the hematological parameters, the PLR was higher in the group of % VO2 max ≤70 than in the group of % VO2 max >70 ($p = 0.003$). NLR values were higher in patients with poor FC, but the difference did not reach statistical significance. Patients with preserved FC had higher LVEF values ($p = 0.003$) and reached a higher peak HR during exercise ($p = 0.006$). CRP, platelet, neutrophil and lymphocyte count, as well as resting HR and blood pressure values, were similar between the two subgroups (Figure 2.9).

Table 2.IV. Univariate analysis of the two groups according to the values of % VO2 max in all study participants.

Parameters	All Patients (n = 80)	% VO2 Max >70 (n = 45)	% VO2 Max ≤70 (n = 35)	p Value *
Age (years) ×	55.51 ± 11.83	57.02 ± 12.08	53.57 ± 11.38	0.19
NLR ×	1.97 ± 0.80	1.83 ± 0.65	2.15 ± 0.93	0.07
PLR ×	155.6 ± 52.7	137.4 ± 35.9	169.8 ± 59.3	0.003
Platelet count, $\times 10^3/\mu L$ ×	256 ± 60	244.4 ± 56.1	266.3 ± 56.1	0.11
Neutrophil count, $\times 10^3/\mu L$ ×	3.32 ± 1.25	2.92 ± 0.90	3.83 ± 1.45	0.001
Lymphocyte count, $\times 10^3/\mu L$ †	1.72 (1.44–1.99)	1.45 (1.31–2.43)	1.86 (1.65–1.88)	0.06
CRP (mg/dl) †	0.41 (0.24–1.04)	0.28 (0.15–1.26)	0.54 (0.26–0.89)	0.82
LVEF ×	51.31 ± 11.04	55.67 ± 9.26	48.71 ± 10.93	0.003
BMI (kg/m^2) †	28.7 (27.4–33)	28.4 (27.4–32.4)	30.15 (25.82–33.17)	0.68
Hypertension □	66 (82.5)	38 (84.4)	28 (80)	0.76
Diabetes □	22 (27.5)	14 (31,1)	8 (22.9)	0.45
HbA1c (%) ×	7.11 ± 1.47	6.58 ± 1.10	7.67 ± 1.66	0.05
LDL (mg/dl) †	84 (69.8–108)	73(69.8-104)	100.8 (56.6–124)	0.57
Resting HR ×	81.9 ± 15.69	84.00 ± 17.25	77.57 ± 12.76	0.05
% peak HR ×	77.98 ± 12.25	82.38 ± 11.21	72.31 ± 11.27	0.001
Resting SBP (mmHg) ×	127.3 ± 12.65	130 ± 13.39	125.3 ± 11.79	0.1
Resting DBP (mmHg) ×	81.5 ± 7.52	80.78 ± 7.305	82.43 ± 7.8	0.33

NLR—neutrophil to lymphocyte ratio, PLR—platelet to lymphocyte ratio, CRP—C-reactive protein, LVEF—left ventricular ejection fraction, BMI—body mass index, LDL—low-density lipoprotein, % HR—percentage of maximal predicted heart rate during test, SBP—systolic blood pressure, DBP—diastolic blood pressure, * Difference between % VO2 max ≤70 and % VO2 max >70. Data are presented as: × Mean ± SD; □ n, %; † Median (interquartile range).

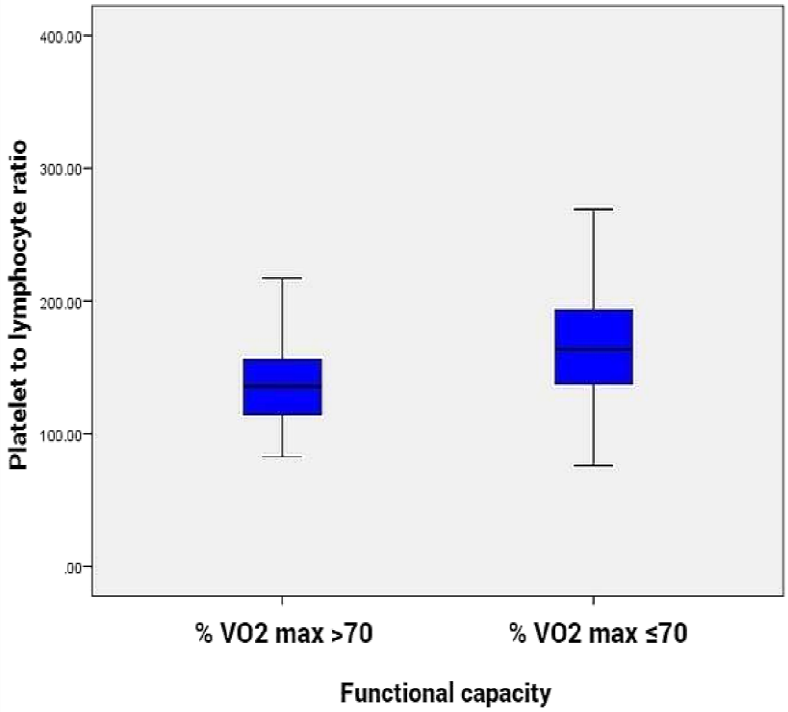


Fig. 2.9. Platelet to lymphocyte ratio levels according to functional capacity groups

PLR were positively correlated with % VO2 max ($p < 0.05$; Table 2.V). NLR was associated with PLR, but not with the analyzed CPET parameters. In a logistic multivariate model, the PLR remained significant predictor of poor FC (Table 2.VI.).

NLR was not a significant predictor of poor FC in univariate analysis; thus, it was not included in the multivariable regression model.

ROC curves explored the relationship between the PLR and FC. Using a cut-off point of 139, the PLR predicted poor FC with a sensitivity of 74% and specificity of 60% (ROC area under curve: 0.681, 95% CI: 0.563–0.799, p = 0.006; Figure 2.10).

Table 2.V. Pearson correlation between NLR, PLR and CPET parameters.

Parameters	NLR	PLR	Resting HR	% Peak HR	% Peak WR	% VO2 Max
NLR	1	0.369 *	−0.087	−0.043	−0.104	−0.133
PLR	0.369 *	1	0.207	0.172	0.105	0.249 *
Resting HR	−0.087	0.207	1	0.594 *	−0.053	0.144
% peak HR	−0.043	0.172	0.594 *	1	360 *	0.448 *
% peak WR	−0.104	0.105	−0.053	0.360 *	1	0.705 *
% VO2 max	−0.133	0.249 *	0.144	0.448 *	0.705 *	1

* $p < 0.05$, NLR—neutrophil to lymphocyte ratio, PLR—platelet to lymphocyte ratio, HR—heart rate, % HR—percentage of maximal predicted heart rate during test, % WR—percentage of the predicted value of maximal work rate, % VO2 max—percentage of the predicted value of maximal oxygen uptake.

Table 2.VI. Multivariate regression analysis to predict poor functional capacity.

Variables	Odds Ratio	95% Confidence Interval	*p*
Neutrophil count, $\times 10^3/\mu L$	1.00	0.999–1.002	0.523
PLR	1.015	1.004–1.027	0.009
LVEF	1.07	1.003–1.141	0.042
% peak HR	1.088	1.029–1.151	0.003

PLR—platelet to lymphocyte ratio, LVEF—left ventricular ejection fraction, % HR—percentage of maximal predicted heart rate during test.

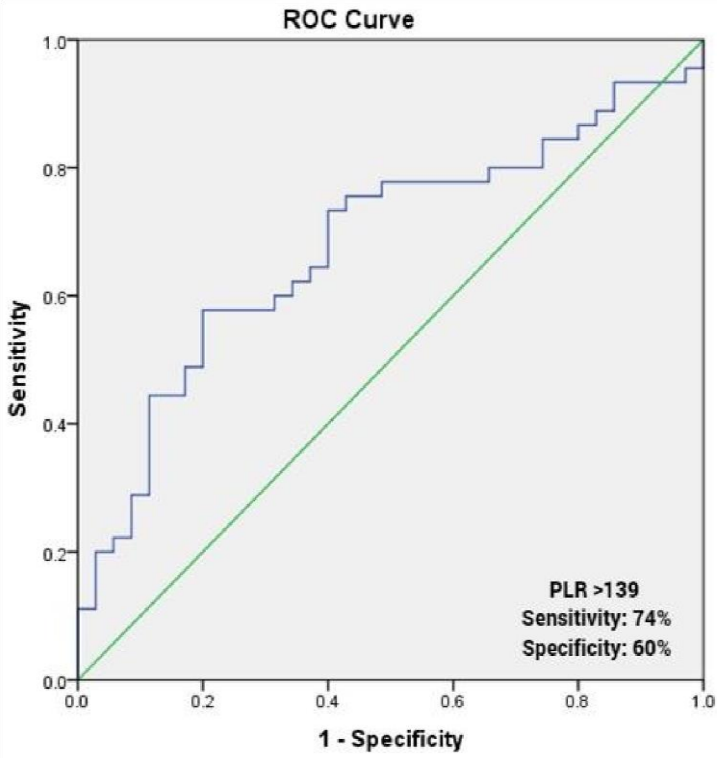


Fig. 2.10. Receiver operating characteristic curve of platelet to lymphocyte ratio for predicting poor functional capacity.

Discussion

The results of the present study suggest that significant prognostic information can be obtained from routine blood test results in CAD patients undergoing CR. *Walzik et al.*

recently published reference values for NLR and PLR, encouraging a more frequent use in clinical practice (Walzik et al, 2021). NLR and PLR were significantly higher among our patients (especially in the subgroup with poor functional capacity) compared to the average NLR and PLR values recently reported in a healthy population-based cohort: 1.76 (0.83–3.92) and 120 (61–239), respectively (Fest et al, 2018).

Functional capacity is an independent prognostic factor in CAD patients (Ambrosetti et al, 2020, Hung et al, 2014; Arena et al, 2007; Vanhees et al, 1994; Coeckelberghs et al, 2016). Our data show that impaired FC assessed with CPET is associated with changes in leukocyte subsets and platelets. Inflammation plays a role in the onset, progression and destabilization of atherosclerotic plaque. Systemic inflammation is known to be associated with parietal vascular inflammation (Libby, 2012). Activation of lymphocytes and monocytes is essential in the early stages of atherosclerosis, while neutrophils are implicated in plaque destabilization and thrombosis (Yayan, 2013). NLR is an easily available biomarker of vascular parietal inflammation (Gary et al, 2013) with documented prognostic implications in various cardiovascular diseases (Afari et al, 2016). Elevated NLR has been associated with an increased risk of atrial (Canpolat et al, 2013; Im et al, 2013; Aribas et al, 2012) and ventricular arrythmias (Chatterjee et al, 2011) and with worse outcomes in acute decompensated heart failure (Uthamalingam et al, 2011) and acute coronary syndromes (Guasti et al, 2011). Besides CAD, NLR offers prognostic information in patients with abdominal aortic aneurysm (Ntalouka et al, 2021), chronic threatening limb ischemia (Russu et al, 2022) and other cardiovascular emergencies (Garagoli et al, 2022; Arbanas et al, 2022; Pasqui et al, 2022; Lee et al, 2020; Efros et al, 2021). NLR is also a biomarker of interest in severe mitral and aortic valvular heart disease (Baysal et al, 2015; Polat et al, 2014) and is a predictor of poor FC in patients with HF (OR 3.085, 95% CI 1.52–6.26, p = 0.002) (Cakici et al, 2014). Indeed, immune dysregulation is known as an important characteristic of poor aerobic capacity. Increased NLR could be associated with poorer physical performance in CAD patients and with lower LVEF in patients with HF (Cakici et al, 2014). In a previous study, Yıldız et al. showed that a threshold level of 2.26 for NLR predicts a poor FC (sensitivity of 83% and specificity of 69%) in patients with idiopathic dilated cardiomyopathy (Yıldız et al, 2015). In another study of 94 patients with compensated HF, NLR was correlated with exercise performance, and a cut-off point of 2.74 was established for predicting poor FC (Cakici et al, 2014). FC was expressed as maximal exercise intensity (METs) during treadmill test in both previous studies, a less specific marker for FC compared to % VO2 max. In the present analysis, although NLR values were higher in patients with poor FC, the difference did not reach statistical significance.

Elevated blood and plasma viscosity have been associated with an increased risk of CAD. CAD patients have increased platelet and monocyte aggregates in their bloodstream, which are associated with plaque instability, worse in-hospital outcomes and an increased risk of future cardiac events (Furman et al, 1998; Zhang et al, 2007). Exercise training improves blood rheology, which may contribute to the increased FC observed after CR (Church et al, 2002). PLR reflects the balance between thrombotic and inflammatory pathways, being influenced by blood viscosity and inflammation (Yayan et al, 2013; Furman et al, 1998). Ayca et al. showed that patients with high PLR had higher Syntax Score (SXS) and a PLR > 137 had a specificity of 52% and a sensitivity of 61% for predicting SXS > 22, marking PLR as a prognostic marker in primary PCI (Ayça et al, 2015). Azab et al. examined the prognostic value of PLR in non-STEMI (Azab et al, 2012). At the 4-year follow up, patients with PLR > 176 had a 42% all-cause mortality, whereas patients with PLR < 118.4 had an all-cause mortality rate of 17%. In another study of patients with STEMI, Ugur et al. found that patients with PLR > 174.9 had higher all-cause mortality at 6 months compared with patients with PLR < 174.9 (Ugur et al, 2014). Moreover, previous studies also showed

that high PLR is associated with increased risks of new-onset atrial fibrillation (Karatas et al, 2016), contrast-induced acute kidney injury (Sun et al, 2018), more advanced HF (Sun et al, 2017) and no reflow after PCI in STEMI patients.

Our analysis shows that PLR is higher in patients with % VO2 max ≤70 than in patients with %VO2 max >70 ($p = 0.003$). PLR was positively correlated with % VO2 max ($p < 0.05$) and remained significant predictor of poor FC (OR, 1.015; 95% CI, 1.004–1.027; $p = 0.009$) after multivariate analysis. Using a cut-off point of 139, the PLR predicted poor FC with a sensitivity of 74% and specificity of 60% (ROC area under curve: 0.681, 95% CI: 0.563–0.799, $p = 0.006$; Figure 2.10).

Other studies have assessed the relationship between CRP and FC in various noncardiovascular conditions (Lopes et al, 2022; Szortyka et al, 2016; Kerget et al, 2021). Our results do not support a significant association between CRP and FC in CAD patients with recent PCI.

The results of the present study suggest that significant prognostic information can be obtained from routine blood test results in CAD patients with recent PCI. Because the PLR is a ratio, it is less prone to bias/variations than individual blood parameters that can be altered by several variables (e.g., dehydration, over-hydration and blood specimen handling). To our knowledge, this is the first study that evaluated whether PLR can predict FC assessed by CPET in stable CAD with recent PCI.

This study has several limitations. Most importantly, this single-center retrospective analysis included a relatively small number of patients and did not include measurement of other important cardiac biomarkers such as troponin and natriuretic peptides. Furthermore, although all patients had a negative COVID-19 PCR upon admission, we were not able to accurately exclude prior COVID-19 infection (we did not perform antibody testing and we did not take into consideration vaccination status).

Conclusions

PLR is higher in patients with recent PCI for stable CAD and poor FC compared to those with preserved FC. FC is an independent predictor of long-term prognosis in CAD. Although CPET is the gold standard test for assessing FC prior to cardiovascular rehabilitation, its availability remains limited. PLR, a cheap and simple test, could predict poor functional capacity in patients with stable CAD and recent elective PCI and help prioritize referral for cardiovascular rehabilitation in high-risk patients.

Previous COVID-19 infection can negatively impact functional capacity and could influence our results. The retrospective structure of our study and the small number of cases, our multiple regression was limited to only a few covariates.

Residual covariates and additional risk factors (for example smoking status) could significantly impact our results. Considering these limitations, our conclusions need to be validated in larger cohort analyses. Furthermore, larger prospective studies are needed to evaluate whether PLR can also predict FC improvement after cardiovascular rehabilitation programs.

I.2.2.3. Oral pathology and diabetes mellitus

Given the fact that I also teach to students of the Faculty of Dentistry, I considered it necessary to deepen the knowledge related to oral pathology. Interrelations between diseases of the stomatognathic system and general pathology are well known.

I.2.2.3.1. Influence of *diabetes mellitus* and smoking on pro and anti-inflammatory cytokine profiles in gingival crevicular fluid

Introduction

Diabetes mellitus and smoking have been recognized as important modifying factors of the evolution of periodontitis, being considered at the moment as descriptive factors in the periodontitis grading system. The aim of this study was to evaluate the effects of type 2 diabetes, smoking, and these two factors combined on gingival crevicular fluid (GCF) levels and ratios of pro-inflammatory and anti-inflammatory cytokines were assessed using a commercially available kit-based multiplex fluorescent immunoassay (Miranda et al, 2019; Liu et al, 2021).

Material and method

The study was carried out on a number of 124 volunteers (control (C) group = 29, Diabetes Mellitus (DM) group = 32, Smoking (S) group = 31 and S + 25 DM group = 32).

Results

Total mean bleeding on probing was significantly lower in the S and S + DM 26 groups compared to the other groups ($p < 0.05$). The total amounts of IL-2, IL-6, IL-17, TGF-β and 27 MIP-1α were significantly higher in the periodontally healthy sites of DM patients ($p < 0.05$), 28 compared to those in the control group. Systemically healthy smoking patients had higher values 29 for IL-4, IL-5, IL-7, TNF-α, TGF-β and GM-CSF, while diabetic smoking patients showed higher 30 values for IL-4, TGF-β and MIP-1α. In smoking and systemically healthy patients, IL-7, IL-12 and 31 IL-23 showed higher concentrations, while concentrations of IL-2, IL-7, IL-12, IL-17, IL-21 , IL-23, 32 MIP-1α and TGF-β were higher in smoking DM patients.

Discussions

This observational study proposed an evaluation of the effects of type 2 diabetes mellitus, smoking and these two factors combined on the inflammatory status in crevicular fluid. Although there are numerous studies in the specialized literature that have focused on the individual impact of smoking and diabetes on cytokine concentrations in periodontal tissues, the association of these two highly modulatory factors remains incompletely elucidated. The results of the present research demonstrated that the presence of diabetes affected the ratios of pro-/anti-inflammatory cytokines, stimulating the overall proportion of pro-inflammatory cytokines and inhibiting the proportion of anti-inflammatory cytokines, showing that diabetes induced a pro-inflammatory state. Diabetes mellitus increased the concentrations of pro-inflammatory cytokines in non-smoking patients, whether or not the sites were periodontally affected, decreased the levels of anti-inflammatory cytokines, and generated higher pro-/anti-inflammatory cytokine ratios. These subjects also showed increased proportions of pro-inflammatory cytokines and reduced proportions of anti-inflammatory cytokines compared to controls. Findings from the present study indicated that diabetes leads to a general pro-inflammatory state in periodontal sites, as also reported in previous investigations (Hao et al, 2023; Graves et al, 2000; Moeintaghavi et al, 2017). Some hypotheses that could explain the pro-inflammatory state of diabetic subjects involve the induction of oxidative stress and the formation of advanced glycation end products (AGEs) by hyperglycemic status in periodontal tissues (Polak et al, 2018). Binding of AGEs to RAGE activates a sequence of cell signaling events, including overproduction of proinflammatory cytokines (Erusalimsky et al, 2021). Hyperglycemic status and AGEs induce reactive oxygen species, leading to continuous formation of AGEs and production of proinflammatory cytokines. Also, an increase in the level of pro-inflammatory cytokines will favor the formation of free radicals (Chang et al, 2023). According to our results, smoking in turn altered pro-/anti-inflammatory cytokine ratios, especially in periodontally affected sites, causing a decrease in the proportion of pro-inflammatory cytokines, indicating that smoking mainly stimulated immunosuppression in periodontal sites. Diabetic smoking patients, however, showed neither the hyper-inflammatory response seen in diabetics, nor

the immunosuppressed response seen in smokers, revealing an intriguing counterbalance of these two risk factors when combined. These data support the hypothesis that diabetes and/or smoking generate significant influences on cytokines, but in distinct ways. It is suggested that changes in cytokine balance induced by these risk factors towards activation or suppression of inflammation may generate negative consequences on periodontal tissues. From the data obtained in the present research, smoking appears to stimulate immunosuppression in periodontal sites rather than an inflammatory status, particularly during the response to periodontal pathogens. In samples collected from smoking patients we observed lower ratios of pro-/anti-inflammatory molecules and higher proportions of anti-inflammatory cytokines than in control subjects. In support of these results, previous studies reported a role of smoking in the reduced manifestation of inflammatory signs (Moeintaghavi et al, 2017; Bunaes et al, 2017; Miranda et al, 2020). An analysis by Zhu et al. (2017) showed that smoking was associated with an ap- proximately 50% increased risk of cardiovascular complications in patients with type 2 diabetes, with the highest point estimate for peripheral arterial disease (combined RR 307 2.15; 95% CI 1.62-2.85). Compared to nonsmokers, ex-smokers also had a moderate 0-20% risk of mortality, chronic cardiovascular disease, but not stroke (Zhu et al, 2017). Several large studies support these observations. In a large retrospective cohort study of 132,462 patients with type 2 diabetes, it was observed that smoking was associated with an increased risk of mortality in both men (RR 1.71; 95% CI 1.56-1.88), as well as in women (2.04 ; 1.58-2.64), during 5 years of follow-up (Wan et al, 2017). Microvascular complications mainly include nephropathy, retinopathy and neuropathy; these complications may be caused by hyperglycemic damage to small blood vessels. Correlations between smoking and microangiopathies have been examined in numerous studies, but the results are not entirely consistent (Tomic et al, 2022). Several biological mechanisms have been postulated to explain the link between smoking and diabetes. However, smoking increases the risk of obesity through an antiestrogenic effect, moreover, abdominal obesity is strongly linked to insulin resistance and diabetes (Driva et al, 2022). Second, nicotine stimulates lipolysis and the release of free fatty acids in the liver and skeletal muscles; these phenomena are associated with increased hepatic secretion of very low-density lipoproteins and intracellular lipid saturation, as well as insulin resistance. Third, smoking increases inflammation and oxidative stress and thus impairs endothelial function, leading to insulin resistance and diabetes, as well as chronic complications such as diabetic nephropathy or periodontal disease (Durlach et al, 2022). In addition, smoking acts on functional nicotinic receptors on pancreatic islets and beta cells, negatively affecting insulin release (Ling et al, 2022). An important observation in the present study was that the diabetes-induced-inflammatory status in periodontal tissues was partially compensated by the action of smoking. Smokers with diabetes showed a trend towards a pro-inflammatory profile in both healthy and diseased periodontal sites, but to a lesser extent than that observed in non-smoking diabetic subjects. Another significant observation was that TGF-β levels were increased, while the ratios of several proinflammatory cytokines and TGF-β were reduced in the periodontal sites of all risk factor groups. TGF-β is a molecule with anti-inflammatory / immuno- suppressive action; increased levels of this molecule in diabetic patients may translate to a failed attempt by the host to control its proinflammatory state, whereas in smokers, increased levels of TGF-β appeared to contribute to sustaining immunosuppression. It is interesting to compare these findings regarding the impact of diabetes and/or smoking on local ratios of pro-/anti-inflammatory cytokines with previous data on the impact of the same risk factors on circulating ratios of pro-/anti-inflammatory cytokines (Santonocito et al, 2022). Previous studies have revealed that periodontitis, when associated with diabetes and/or smoking, is corelated to the overall systemic proinflammatory burden. Thus, it is hypothesized that

diabetes and/or smoking in subjects with periodontitis alter periodontal and circulatory cytokine profiles in distinct manners (Barutta et al, 2022). A single cytokine molecule may generate modest activity during the host's inflammatory response to periodontal pathogens; its effect is, however, potentiated by its interaction with other cytokines. It has been speculated that this pattern of cytokine clustering and the increased individual ratios of pro-/anti-inflammatory cytokines observed in healthy sites of diabetic subjects may contribute to placing these sites in the risk zone for periodontitis (Kozak et al, 2023). Moreover, these complex cytokinin networks should be explored in other contexts of periodontal disease and other systemic diseases and conditions and periodontal disease treatments (Maftei et al, 2021; Martu et al, 2021; Budala et al, 2023; Mocanu et al, 2021; Martu et al, 2021; Luchian et al, 2021; Zhang et al, 2023). We also observed that six molecules (three anti-inflammatory [IL-4, IL-5 and IL-13] and three pro-inflammatory [IL-12, IL-21, IL-23]) were closely related in the diseased sites 361 of to all evaluated groups, regardless of risk factors. Thus, diabetes appears to significantly influence cytokine interactions in healthy sites; however, with the presence of periodontal involvement, cytokine relationships were influenced by periodontal infection rather than risk factors. Moreover, in addition to the studied cytokines, a number of other mediators may be involved in the periodontal destructive inflammatory phenomena in diabetic smoking patients. Further investigations focusing on the intersecting effects of diabetes and smoking on the etiopathogenesis of periodontitis are needed.Our study draws attention to the need for urgent attitudes and actions for smoking cessation as a crucial component for the prevention and management of diabetes, especially in patients with periodontal disease.

Conclusion

In our study the proportion of pro-inflammatory cytokines was higher in the group of non-smoking patients with diabetes and lower in the group of smoking patients. The proportion of anti-inflammatory cytokines was lower in both diabetic groups and higher in the smoking patient group compared to the control group. Diabetes mellitus induced a general proinflammatory state, while smoking mainly stimulated immunosuppression in the periodontal tissues of periodontitis subjects.

I.2.2.3.2. Risk factors for periodontal disease in diabetic patients

Introduction

Periodontal disease is one of the most widespread chronic illnesses with a huge negative impact on the quality of life. Its prevalence and severity increase with age, chronic periodontal inflammation causing gingival bleeding, periodontal pockets and alveolar bone loss (Martu et al, 2004).

The relationship between the periodontal disease and other age-related systemic diseases has been increasingly discussed, those conditions being associated with increased inflammation markers and epigenetic modifications in the regulation of gene expression leading to molecular modifications (Mark et al, 2000). Also, many of these inflammatory diseases are sharing common risk factors such as age, smoking, obesity, stress, physical inactivity, environmental factors, metabolic syndrome, systemic medication and nutritional factors (Joshipura et al, 2010). Several studies indicate a direct relationship between periodontal disease and diabetes mellitus (Bray et al, 2002).

The common physiological mechanism is inflammation, periodontitis occurring in an exacerbated host response with hyperproduction of mediators (Deshmukh et al, 2011).

In addition, hyperglycemia causes alteration of oral microbial flora, vascular alterations and collagen metabolism changes which may promote the onset or the worsening of periodontal disease (Gurav et al, 2011).

Material and methods

To achieve our purpose we conducted a prospective study in 117 patients admitted to the CF Hospital Iasi, Clinic of Internal Medicine and Geriatrics-Gerontology. 72 patients formed the study group being diagnosed with diabetes mellitus based on the repeatedly elevated blood glucose levels > 126 mg / dl.

The control group consisted of 45 patients hospitalized with other medical conditions than diabetes such as angina, hypertension, kidney stones, chronic bronchitis or rheumatic conditions. All patients were medical and dental evaluated and they were asked to fill out questionnaires on demographic data, oral symptoms and risk factors associated with periodontal disease. All the patients were consulted in the dental cabinet of CF Policlinic using the dental consultation kit (dental mirror, dental probe and dental clamp).

The diagnosis of various forms of periodontal disease was based on clinical and laboratory signs acquired from the subjects: changes in color and consistency of the gums, gingival bleeding, spontaneous or provoked pain, gingival retraction, periodontal pockets of various depths, bone lysis observed on X-ray.

Exclusion criteria included severe diabetic complications, renal insufficiency, other systemic diseases considered as risk factors for periodontal disease, antibiotic treatments over the past 6 months, antihypertensive calcium antagonists, antiepileptic drugs, steroids or immunosuppressive drugs and periodontal treatments over the past 6 months.

The two groups were comparable regarding age, sex distribution, level of oral hygiene (assessed by questionnaires), the percentage of smokers and obese patients. Recorded data were analysed statistically, a value of p statistically < 0.05 being considered statistically significant.

Results

The present epidemiological study includes 117 patients, 83 males and 34 females with a mean age of 60 years. Age, gender, and environment distribution were similar for the study and control group. The prevalence of periodontal disease in all patients was found to be 73.50%. In the study group almost all patients (69 from a total a 72) had different types of periodontal disease (95.83% respectively), while in the control group only 17 out of 45 non-diabetic patients (37.77%) suffered from periodontal disease. Analysing the prevalence of periodontal disease in age groups, we found that the periodontal disease prevails after the age of 65, reaching 100% in elderly diabetics compared with 43.75% in elderly control patients (Figure 2.11).

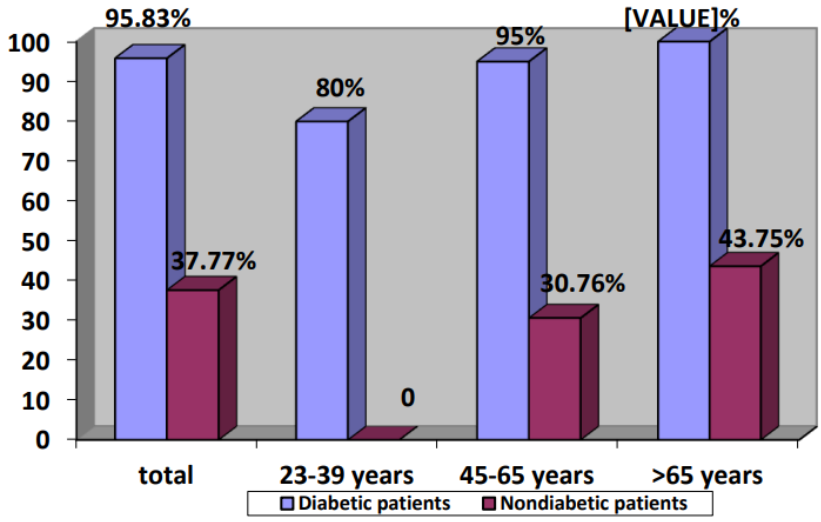


Fig. 2.11. Prevalence of periodontal disease in age groups patients

According to gender, the periodontal disease was more common in both groups in male patients, but it was significantly higher in diabetic men reaching 100% compared to men in the control group where the prevalence of the disease was 60%.

Analysing the prevalence of periodontal disease in diabetic patients based on the type of diabetes, we found that it reached 77.77% among type 1 diabetics, less than in type 2 diabetes. Patients with type 2 diabetes were diagnosed with periodontal disease in a significant percentage of 97.43%.

This difference is probably due to the young age of patients with type 1 diabetes, while patients with type 2 diabetes belonged in the age groups 40-65 years and especially over 65 years.

Additionally, at more advanced ages we can expect a longer evolution of diabetes and the presence of more complications with possible implications for periodontal disease (Figure 2.12).

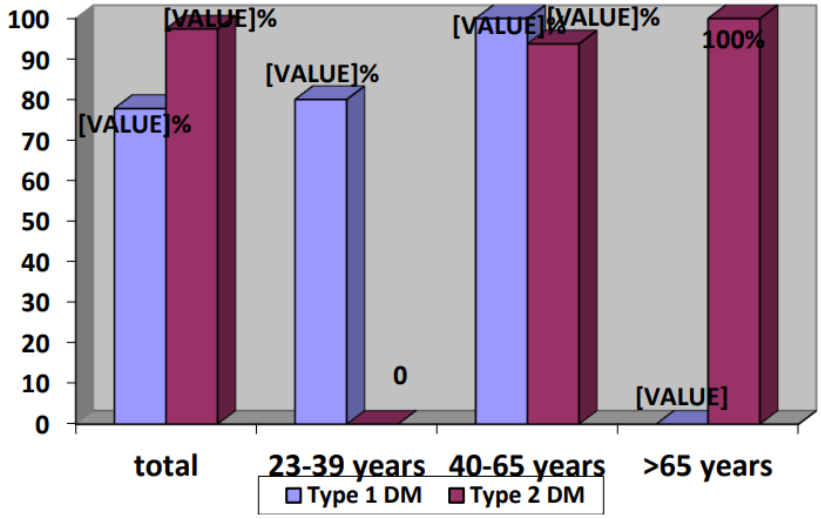


Fig. 2.12. Prevalence of periodontal disease in age groups patients depending on the type of diabetes

Discussions

In order to assess whether diabetes can be considered as an independent risk factor for periodontal disease we evaluated the association of other risk factors such as home environment, smoking, obesity and oral hygiene. Analysing the prevalence of periodontal disease according to the home environment, we noticed that the periodontal disease was prevalent in the rural patients in both studied lots, but while the control group prevailed 58.33%, the diabetic rural patents were all diagnosed (100 with periodontal disease.

The prevalence of periodontal disease in smokers was 79.03% compared to 67.27% in non-smokers, demonstrating the importance of smoking as a risk factor. However, we noted that in non-diabetic patients the presence of smoking has significantly increased the prevalence of periodontal disease from 23.80% to 50%. In the study group the prevalence of periodontal disease was extremely high in both smokers and non-smokers (97.36% vs. 94.11%), advocating the role of diabetes as an independent risk factor.

The prevalence of periodontal disease in obese patients was 94.33% compared to 56.25% in normal weight patients demonstrating the importance of obesity as a risk factor. In non-diabetic patients the presence of obesity has increased the percentage from 11.11% to 77.77%.

In non-diabetic patients the presence of obesity has increased the percentage from 11.11% to 77.77%. In diabetic patients the prevalence of periodontal disease was higher than in the controls in both subgroups with obesity (97.14% vs. 77.77%) and especially in the normal weight patients (94.59 % vs. 11.11%). The difference between the prevalence of

periodontal disease in weight subgroups in diabetic patients was significant but less important compared to the differences between these subgroups in non-diabetic patients, which supports the role of diabetes as an independent risk factor (Figure 2.13).

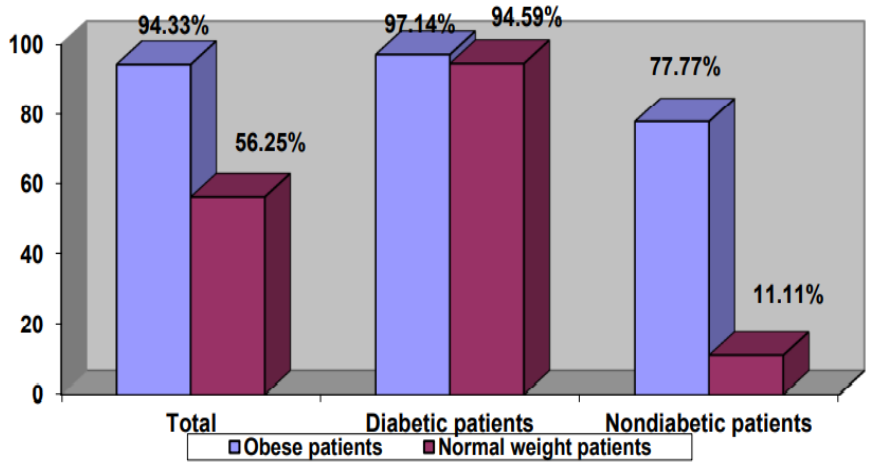


Fig. 2.13. The prevalence of periodontal disease in obese/non-obese patients

Periodontal disease was present in all diabetic patients regardless of oral hygiene quality while in non-diabetic patients only those with poor oral hygiene were affected. Diabetic patients with poor hygiene had a prevalence of periodontal disease of 100 % while patients with other conditions than diabetes were affected in 87.5%, much more than those with a good oral hygiene (14.28%) (Figure 2.14).

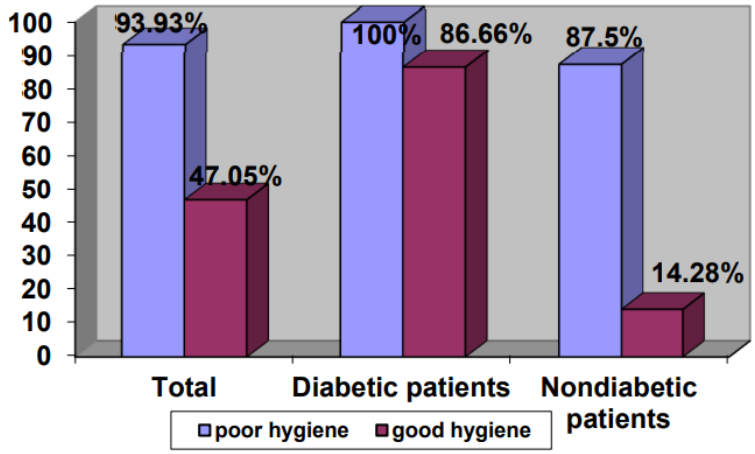


Fig. 2.14. The prevalence of periodontal disease according to hygiene oral status

Conclusions

The results of the present study demonstrated that periodontal disease is more frequent and pronounced in diabetic patients than in controls especially in type 2 diabetes, over 45 years age group, males from rural environment. However, we noted that the presence of smoking, obesity and poor oral hygiene have increased the prevalence of periodontal disease predominantly in non-diabetic participants which supports the role of diabetes as an independent risk factor.

The diabetic patients must be informed on the risk of periodontal disease and the clinician should encourage them to improve their oral health and to schedule regular visits to the dentist as an important component of their overall diabetes management.

I.2.2.3.3. The role of chemical factors in the diabetes and the prediabetes that leads to polymorph oro-maxillofacial alterations in malformative syndrome

Introduction

Diabetes mellitus is the most important metabolic disease with clinical manifestations and multiple biochemical alterations, which leads to buccal-maxillo-facial polymorphic alterations. The malformation syndrome covers a large variety of malformations produced by different diseases, environmental status or teratogenic drugs. There are also many psycho-emotional factors that interfere with and causes foetal damages. Periodontal lesions are disturbing or distressing inflammation hyperplasic tissues of periodontium and producing both gingivitis and periodontitis. The most important factor in the epidemiology of the periodontal disease for prophylaxis is oral hygiene (American Diabetes Association, 2005; Armstrong et al, 2005).

Material and methods

The study group consisted of 90 patients with congenital diabetes and stomatological problems, aged between 26 and 65 years, the average age being 45 years. Distribution by gender: 60 men and 30 women.

Results

Ninety cases of diabetes, malformations and dental problems were studied. Saliva plays an important role in preventing periodontal disease, but its actions are limited to clinical crowns: those parts of the teeth and gums that are exposed to saliva. These places are part of the salivary field of the host defence, while the gingival sulcus and periodontal pockets are in the crevicular field because the host defence. In these places it is mediated by crevicular fluid and inflammatory cells. A major function of saliva is to be a vehicle for swallowed bacteria and other debris from the oral cavity. Saliva bacteria colonize teeth. It averages 108 bacteria per mL, their number dropping after the meal and then slowly rising again.

Microorganisms multiply on the dorsal face of the tongue and the plate and are transferred into saliva by food and soft tissue movements. Then they are quickly removed by swallowing. Salivary antibacterial actions are: a vehicle for swallowed bacteria; inhibition of bacterial attachment; bacterial aggregation in saliva; bacterial killing through the peroxidase system; killing bacteria by lysozyme, lactoferrin and other factors. In addition to its antimicrobial activity, saliva, paradoxically, also contributes to plaque formation. It is a rich culture medium for those microorganisms that are adapted to live in the oral cavity and the initial colonization of the plaque.

Discussions

Diabetes saliva contains a variable but not increased amount of glucose, which explains the high frequency of oral cavity infections in these patients (Nash et al, 2008; Popescu et al, 2013; Ciurcanu et al, 2016; Scutariu et al, 2016). The specific immune system is represented in the saliva and mucosal surfaces by the secretory immune system. Unlike the humoral immune system, the secretory system produces Ac-IgA in response to mucosal surface antigens. Microorganisms in the oral cavity stimulate the system in the gut after they have been swallowed and activated lymphocytes then migrate into the salivary and secreted IgA glands.

Saliva contains a non-specific antimicrobial peroxidase system consisting of salivary peroxidase enzymes, hydrogen peroxide and thiocyanate ions. The system is analogous to the myeloperoxidase system used by neutrophils to kill microorganisms. Peroxidases are synthesized by salivary glands and secreted in saliva where they bind to bacteria, and thiocyanate is secreted into saliva by duct cells. Hydrogen peroxide is continuously generated in the oral cavity at very low concentrations by bacteria, neutrophils and other

host cells and is used by peroxidases to oxidize thiocyanate to hypothiocyanic acid that kills bacteria. However, although the salivary peroxidase system kills bacteria in saliva, it does not appear to inhibit platelet bacteria or periodontal pockets (Botnariu et al, 2017; Ifteni et al, 2016; Jumanca et al, 2014).

Lysozyme is another antimicrobial enzyme in saliva and is mostly secreted by mucous salivary glands with a small contribution secreted into the cervical fluid by neutrophils. Saliva is very important in preventing excessive overgrowth and its importance is clearly demonstrated by xerostomia. The epithelial barrier function, is a major function of the oral mucosa, is to prevent the entry of bacteria and their products into tissues. In health, the junction epithelium provides sealing against bacteria around the teeth and is permeable as a result of its adaptation to attachment but is poorly adapted to preventing the migration of bacterial products into tissues (Balan et al, 2017; Zegan et al, 2015; Ancuta et al, 2018).

With the disease, it proliferates to form the epithelium of the bag, retaining its great permeability and becoming interrupted and ulcerated. In addition to barrier function, the epithelium also has important roles in initiating and maintaining immune and inflammatory responses. Since the epithelium can induce both immune and inflammatory response, it is likely that bacteria and their plaque products will induce gingivitis by damaging the epithelium, sooner than through diffusion into connective tissue (Balan et al, 2017; Zegan et al, 2015; Ancuta et al, 2018).

Clinical signs of gingivitis are visible after a few days accumulation of the plaque, but the microscopic evidence of inflammation is always present, even in apparent health. Periodontitis is a classic example of chronic inflammation and those features that are not part of typical inflammatory reactions such as epithelial detachment, bag formation and tooth loss result from the particular anatomy of periodontitis. Inflammation is the fundamental response of living tissues to aggression and develops a first line of rapid defense against lesion and infection. The functions of the inflammatory response are to reduce or eliminate the agents that cause the lesion and, if possible, to destroy them (Budacu et al, 2017; Nemtoi et al, 2017; Ancuta et al, 2018).

Since the microbial plate cannot be completely removed by host defense, the inflammatory response, which involves fluid exudation and neutrophil migration, occurs in the gum just for a short period in the early stages of the disease.

Decreased salivary flow is more important in diabetic patients who have a history of multiple infections. These disorders entail the salivary flow dynamics and other injuries such as fissure lips, dry jugular mucosa, red, fissure, bulky tongue, and dry mouth sensation causing great discomfort, and is characteristic of insufficiently controlled diabetes, where glycolysis hemoglobin exceeds 13%. Oral fluid rich in glucose and a low leakage rate multiplier provides ideal conditions for microbial species aggressive, high antibacterial activity is considered by most specialists as the main cause of periodontal dental pathology.

Conclusions

Since the earliest times (Hippocrates), the interdependence between oral pathology and general pathology has been reported. The human organism is now regarded as a unitary one and, given the increased frequency of oral manifestations during the onset and evolution of general affections, we can deduce the practical importance of oral pathological manifestations in diagnosis, prognosis and therapeutic attitude in internal diseases.

The resonance of diabetes mellitus on the oral cavity in patients with malformations is significant, affecting all components of the dental system.

The congenital abnormalities may be genetic in origin, or environmental infectious, although in most cases it is difficult to identify their aetiology. The oral cavity hygiene status is a decisive factor in any prosthetic treatment, especially in patients with diabetes that have a low body resistance.

I.2.2.3.4. Chemical factors which prompt oral pathological phenomena in some nutrition diseases

Introduction

Diabetic microangiopathy is a condition of the vascular terminal package (arterioles, capillaries, venules) with a preferential location in the kidney, retina or vasorum vessels. Microangiopathy is found in all stages of diabetes: it is established early and is present especially in the stages of latent diabetes; it is considered a symptom rather than complication; it is especially common in young patients (Hamman et al, 2006; Hancu et al, 2012; Botnariu et al, 2018). Parotid hypertrophy is the result of hypersecretion of antiinsulin hormone, producing the same disorders as insular failure by blocking insulin action (Mindruta et al, 2014; Cuciureanu et al, 2016). Diabetic gingivitis concerns only the periodontium of the cover, without including the alveolar ligaments and the alveolar bone. Chronic superficial periodontitis is very common in younger age, considered as an early manifestation of the disease. Hyperglycaemia is the expression of decreased intracellular glycolysis. It can lead to the periodontal condition due to the reduction or inactivation of insulin; it is responsible for the tendency to chronic infections in periodontium. Diabetes creates in the alveolar bone favourable conditions for the internal development of the alveolar pyorrhoea in the form of an osteoporotic atrophy process (Jarvela et al, 2006; Kim et al, 2011). Low body resistance determines periodontal complications such as gingival-periodontal abscesses. Diabetic stomatitis is characterized by dry mouth sensation, gingival pruritus, oral pyrexia, gingivorrhagia, stings and pruritus at the anterior 1/3 level of the tongue, acetone smell. The oral mucosa is bright red, with gingival ulcerated edges, interdental papillae increased in volume and bleeds easily. Ulcero-necrotic stomatitis may worsen the disease, but diabetes can also cause ulcero-necrotic stomatitis. Another manifestation encountered in the diabetic patient is the cheilitis characterized by dry lips covered with squamous areas, with commissural ragas. There is an increased frequency of glossopathy, in the sense that the tongue is enlarged by volume, grooved by trenches, hyperemiated fusiform papillae. In other cases, the tongue is flaccid, atonic, with pain, burns, tingling and pricking. We can also find gangrene injuries. Jugular mucosa leucoplakias have the appearance of a white-grey patch; dental and abundant tartar, alveolar crest atrophy are other manifestations encountered. Post-surgical haemorrhages are also encountered due to the increase of vascular permeability and fragility (Knowler et al, 2009; Raftu et al, 2018). The trigeminal neuralgia encountered in diabetes patients has the following characteristics: migrating pain, bilateral pain; night exacerbation; disappears after administration of insulin. Sometimes we can also find thrushes, mycotic stomatitis, candida albicans, etc.

Material and methods

The research resulted in the following: out of the total of 165 cases, 102 cases (61.81%) presented diabetes, 34 cases (20.62%) obesity, 29 cases (17.57%) gout. The cases studied were represented by 112 women (67.87%) and 53 males (32.12%). The frequency of disorders has increased after 40 years, and the highest number of cases has occurred between 50 and 60 years. Oro-dental changes have been frequently encountered in diabetic and obese patients in much greater proportions than in healthy controls or with other diseases.

Results

Following the study made on 165 patients with orodental manifestations, the following findings were made: anamnesis, research of personal and heredo-collateral history are mandatory stages that every dentist must undergo in front of susceptible, oro-dental lesions, supplemented by laboratory exploration; re-balancing the diabetic is an indispensable thing for local treatment, local anaesthesia with adrenaline is contraindicated, being hyperglycaemic and necrotizing; salivary flow disorders are present in over 90%; oral

mucosa exhibits more or less characteristic changes: erythematous stomatitis 13%, ulcerative-necrotic stomatitis 15%, mycotic stomatitis 2%, glossitis represented 37%, glossodynia 41%, periodontitis 38%; 94 cases presented tartar, dental caries 34%, parathyroid hypertrophy 6%, angular cheilitis 34%, pain syndrome 24%. The extensive and total edentations were frequently encountered as well as the atrophy of the alveolar ridges due to the loss of teeth through periodontal diseases. 34 cases (20.62%) presented obesity. Oro-dental disorders were: sialorrhea 11 cases (6.66%), hyperorexia 9 cases (5.45%), glossitis 13 cases (7.87%).

The patients with acute gout include 29 cases with acute gout in the chronic stage. They presented joint manifestations - 21 cases (12.72%), acute gout attack - 4 cases (2.42%), oral form - 2 cases 12.12%), uric nephropathy-2 cases (12.12%).

Oral syndrome consisted of hyposalivation and xerostomia in the acute phase of gout with multiple caries. One of the cases had a buccal debut represented by temporo-mandibular arthritis, xerostomia, pharyngitis, dysphagia, red oral mucosa, difficulty in swallowing. The teeth are easily crushed. The abrasion is very early, sometimes the submaxillary glands are enlarged. 11 cases (7, 33%) had gouty arthritis presenting joint attacks.

During the algic phases, the patients had salivary hypersecretion, xerostomia, hyperesthesia and dental neuralgia, increased salivary viscosity, with sodium urate crystals in the saliva. The tempo-mandibular joint was very painful, with tendency to trimus (4 cases - 3.03%).

Discussions

Chronic marginal superficial periodonopathy is found characterized by jawbone tension, papillary swelling, dental mobility, serous secretion in the pockets (WHO, 2006; Wucher et al, 2010). There were 14 patients (8.48%) with erythematous stomatitis and non-painful hyperkeratosis plates located in the gingival-jugal ditch. The lesions in the oral cavity must be seen in the light of systemic pathological processes and correlated with systemic illnesses because systemic diseases produce oral manifestations and vice versa, oral disorders produce systemic manifestations.

Conclusions

Considering the human organism as a unitary whole and considering the increased frequency of oral manifestations during the onset and evolution of general disorders, the practical significance of oral pathological manifestations in the diagnosis, prognosis and therapeutic attitude in internal diseases results. General pathology influences and is, in turn, influenced by oral pathology. Oral manifestations can hold the primary role in establishing early diagnosis in a serious condition that can be improved by appropriate therapy.

I.2.2.3.5. IL 6 is correlated with metabolic syndrome parameters in oral lichen planus

Introduction

Lichen planus (LP) is a common chronic inflammatory disorder of unclear etiopathogeny affecting 0.1-2% of the general population. Oral mucosal involvement is present in 70-77% of LP patients and may represent the only manifestation of the disease. It is currently considered a complex immunological disorder, potentially correlated with metabolic syndrome, systemic inflammation, and cardiovascular risk (Davidovici et al, 2010; Dreiher et al, 2009; Arias-Santiago et al, 2011).

Thus, the concept of potentially protective mediators (antiinflammatory) and proinflammatory mediators (proatherogenic) was developed. The first category includes: leptine, adiponectine, IL10, and the second includes: S100 protein, TNF-α, IL1, IL6, IL8, IL12, IL15, IL17, IL18, IL20, IL23, IFNγ, MCP1, MMPs (MMP9), CRP, PAI-1, TSP-1,

MIF, M-CSF, sPLA2-IIA (Dreiher et al, 2009; Arias-Santiago et al, 2011; Tanaka et al, 2014). Detection of abnormal serum concentrations of proinflammatory or proatherogenic factors such as IL6 in patients with oral lichen planus (OLP) may be an argument in favour of screening these patients for metabolic syndrome, as well as a reason for a therapeutic strategy that targets IL6, strategy that was already proven efficient in other inflammatory conditions (Arias-Santiago et al, 2011). The correlation between IL6 and metabolic syndrome parameters in patients with oral lichen planus has not been studied yet, to our knowledge.

Material and methods

We conducted a prospective study on 36 patients consulted in the Dermatology Clinic of CF University Hospital Iasi, Romania during August 2014-January 2015, divided into 2 groups: group I (OLP patients) - 18 patients with oral lesions of lichen planus according to the WHO criteria for pathological diagnosis and group II (control group) - 18 patients with other oral inflammatory conditions: oral candidiasis (8 patients), marginal exfoliative glossitis (3 patients), aphtous stomatitis (2 patients), burning mouth syndrome (2 cases), mucocelle (2 cases), bullous erythema multiforme (1 patient).

Only patients with no systemic treatment (corticosteroids, retinoids, immunosupressants) for at least 6 months prior to consultation were included. Serum IL6 and insulin levels were analysed on blood samples drawn without anticoagulant, centrifuged and freezed at – 80^{o}C on an automated IMMULITE 2000 (Siemens) machine using a chemiluminescence technique. Peripheral insulin resistance was assessed by HOMA-IR index (Homeostasis Model Assessment of Insulin Resistance), calculated using the formula: [(*à jeune* insulinemia X *à jeune* glicemia): 405].

Metabolic syndrome markers: lipid profile (serum triglycerids, total cholesterol, HDL-cholesterol) and blood glucose were analyzed using DIASYS (German Diagnostic System GmbH) commercial kits on an automated CS-800 machine. For the statistical analysis we used SPSS 17.0 software.

All patients signed an informed consent and the study was approved by the ethics committee of the "Gr. T. Popa" University of Medicine and Pharmacy Iasi.

Results

Statistical analysis revealed a significantly higher frequency of female patients in OLP group (83,3% vs 50%; p=0,036), for whom the relative risk of acquiring OLP was 1,67 higher (RR=1,67). The age of all patients varied greatly, between 21-78 years, with an average of 52,53±14,38 years and a median of 55 years. In the OLP group we found an average age slightly higher compared to the control group (55,33 vs 49,72 years).

The most common clinical forms were erosive and atrophoerosive: 10 cases (55,5%). Two of these patients fulfilled the diagnostic criteria for Grinspan syndrome (OLP, arterial hypertension, type 2 diabetes mellitus) and Hewitt-Pelisse syndrome respectively. Chronic hepatitis with C virus associated with erosive OLP was present in 4 cases (22 %). Other clinical forms were: reticular OLP – 5 cases (27,7%) and plaque OLP - 3 cases (16,6%).

History of the oral lesions of lichen planus was between 2-8 weeks (reticular form), 3-6 months (plaque OLP) and 8-30 months (erosive OLP). IL6 serum levels varied from individual values <2 pg/ml up to 15,30 pg/ml. 33,3% of OLP patients versus 5,6% of the patients in the control group had pathological values of serum IL6. Median IL6 values were significantly higher in OLP patients (5,66 vs 3,40 pg/ml; p=0,05) (Figure 2.15).

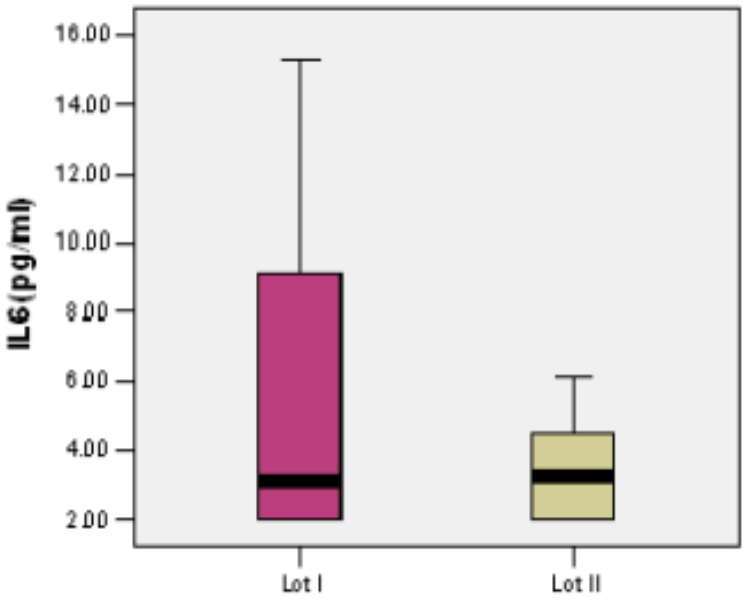
In OLP patients the average serum levels of IL6 were significantly higher in female patients (5,96 vs 4,10 pg/ml; p=0,05) and at ages over 55 years (7,26 vs 4,37 pg/ml; p=0,006). In group II there were no significant differences between sexes (3,12 vs 3,67 pg/ml; p=0,417) or age groups (3,24 vs 3,71 pg/ml; p=0,507) of IL6 average values. In OLP

patients insulin serum levels were slightly more elevated in women (19,01 vs 13,79 µ UI/dl; p=0,573) and significantly higher at ages over 55 years (21,40 vs 15,53 µ UI/dl; p=0,049).

Serum levels of IL6 and insulin directly correlated but with moderate intensity, over 30% in the OLP group and 12,5% of the patients in group II associating high levels of IL6 and insulin, but the result cannot be extrapolated to general population (p>0,05) (Figure 2.16).

Blood glucose levels varied from 74 to 204 mg/dl. Individual levels higher than normal were registered in 27,8% of OLP patients and 16,7% of group II patients.

Median values were slightly higher in patents with OLP compared to group II (109,50 vs 97,44 mg/dl; p=0,289). IL6 directly correlated with blood glucose level, 30,3% of OLP



patients (p=0,222) and 48,4% of group II patients (p=0,048) associating high levels and glucose and IL6 (Figure 2.17).

Fig. 2.15. Median serum levels of IL 6 for the two study groups, comparatively

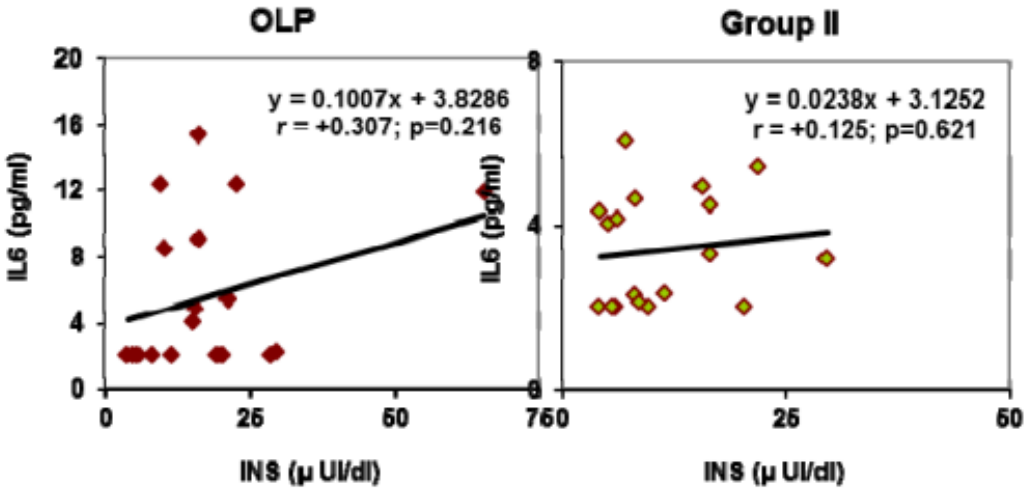


Fig. 2.16. Correlation between IL 6 and insulin serum levels in the 2 groups

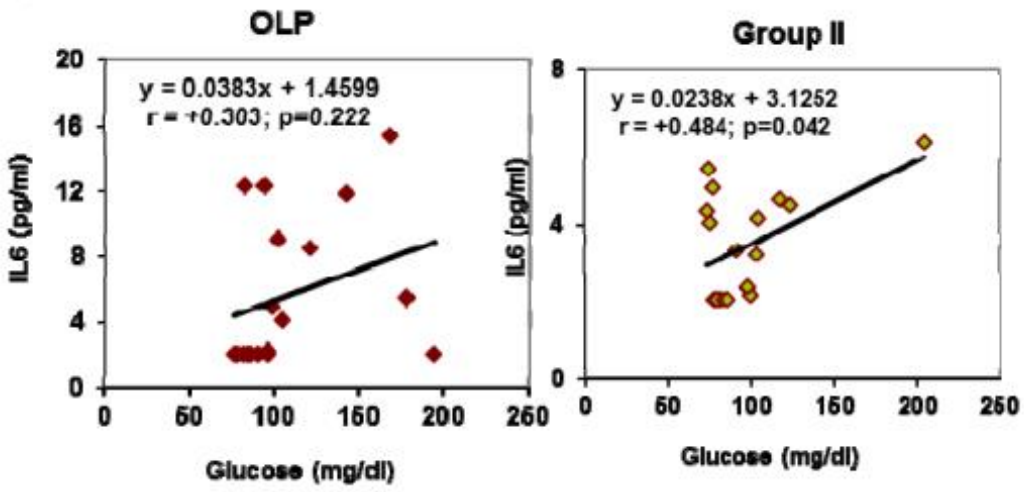


Fig. 2.17. Correlation between IL 6 and serum glucose levels in the two groups

Discussions

The link between LP, the metabolic syndrome and cardiovascular morbidity risk is well documented (Davidovici et al, 2010; Dreiher et al, 2009; Arias-Santiago et al, 2011). Recent studies document the impact played by chronic extravascular inflammation with regards to cardiovascular disease.

Several inflammatory cutaneous morbid conditions including psoriasis, androgenetic alopecia, acne inversa, hidradenitis suppurativa, systemic lupus erythematosus, molluscum fibrosus, acanthosis nigricans and lichen planus have been found to associate with cardiovascular risk factors such as decreased glucose tolerance, dyslipidemia, high blood pressure, obesity and hyperinsulinemia (Davidovici et al, 2010; Dreiher et al, 2009; Arias-Santiago et al, 2011; Bonora et al, 2002).

Hiperinsulinemia, on the other hand, may be considered a cause of secondary dyslipidemia, arterial hypertension and atherosclerosis because of insulin peripheral receptors resistance that lead to metabolic syndrome. It was thus proposed that HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) be added to the criteria of metabolic syndrome as defined by NCEP (Davidovici et al, 2010; Dreiher et al, 2009; Arias-Santiago et al, 2011; Bonora et al, 2002).

IL-6 is a cytokine that promotes specific humoral and cellular immune reactions by stimulating the terminal differentiation of B cells into immunoglobulin-producing plasma cells and CD4+ cell specific differentiation of naive effector CD4+ T cells (Gabay, 2006; Tanaka et al, 2014; Kishimoto et al, 2005; Grossman et al, 1989; Scheller et al, 2011).

Thus, together with TGFβ, IL6 participates in CD4+ cell differentiation of Th17 effector cells antigen - specific pathogen will lead to the elimination of regulatory T cells and inhibits what correlates with suppression of immune tolerance and subsequent development of autoimmune and inflammatory reactions. The early inhibition of IL6 in autoimmune disease models, led to the disappearance of the dominance of Th17 and Th1 over regulatory cells and suppress autoimmune disease (Grossman et al, 1989; Scheller et al, 2011; Paquet et al, 1996; Kishimoto et al, 2006; Xing et al, 1998).

The persistence of proinflammatory activity of IL-6 leads to a switch from acute to chronic inflammation, thus evolving the cytokine role in perpetuating inflammation and favoring acute inflammatory transition to chronic inflammatory infiltrate in lesional tissue area through a continuous secretion of chemotactic protein for monocytes (MCP-1), angioproliferative and antiapoptotic action on T cells (Gabay, 2006; Kapanski et al, 2003). These events are accompanied by a high level of serum IL-6.

Disorders which exhibit high serum levels of IL-6 are rheumatoid arthritis (RA), systemic juvenile idiopathic arthritis, psoriasis, Crohn's disease, lupus, ankylosing spondylitis (Paquet et al, 1996; Kishimoto et al, 2006; Barnes et al, 2011).

Abnormal IL-6 levels can be found in synovial fluid of RA in the lymph nodes of Castleman's disease, myeloma cells, peripheral blood cells or tissues of patients with other diseases and in tumor tissue (Paquet et al, 1996; Kishimoto et al, 2006; Barnes et al, 2011).

Conclusions

The present study aimed to determine a possible relation between OLP, systemic inflammation and metabolic syndrome. Could this dermatosis with a chronic evolution, in a manner similar to psoriasis be a marker for systemic inflammation? Our study shows that there is a significant correlation between systemic inflammation and metabolic syndrome in oral lichen planus.

However, our study groups are small and future studies in larger cohorts of patients with OLP are needed, taking into account several other cytokine promoters of inflammation, on one hand to clarify the pathogenesis of the disease and, on the other hand, to uncover new therapeutic targets that could also embody a preventive strategy of cardiovascular comorbidities in these patients.

I.2.2.4. Other oral pathologies

I.2.2.4.1. Colchicine in the treatment of refractory aphthous ulcerations

Introduction

Colchicine is a traditional natural remedy known for more than a millennium for its anti-inflammatory properties. Biochemically, it is a toxic protoaloalkaloid from the group of tropolon derivatives, that was extracted in 1819 from the bulb of the autumn crocus (*Colchicum autumnale*), and in 1833 was named colchicine by the German pharmacist and chemist Philipp Lorenz Geiger (Karamanou et al, 2018; Nerlekar et al, 2014; Robinson et al, 2018).

The pure crystallized form was obtained by the French pharmacist Alfred Houde (Karamanou et al, 2018). The current drug is the same purified natural substance. The mechanism of action is still a research topic today. In the years 1950-1960, the main cellular target of colchicine action, the cytoskeleton, was identified. Microtubules are major constituents of the cytoskeleton with a role in cell dynamics, maintaining cell shape through resistance to compression, intracellular transport and cell division (Robinson et al, 2018; Leung et al, 2015; Slobodnick et al, 2015; Slobodnick et al, 2018; Paschke et al, 2013).

Colchicine binds to tubulin heterodimers, constituents of protofilaments in the structure of microtubules, to form dimer-colchicine complexes that attach to the end of microtubules causing alterations in their conformation resulting in altered cell function (Robinson et al, 2018; Leung et al, 2015; Slobodnick et al, 2015; Slobodnick et al, 2018; Paschke et al, 2013).

Its therapeutic action is attributed to the inhibition of neutrophil chemotaxis, their adhesion and recruitment in inflammatory lesions as colchicine is more concentrated in leukocytes than in plasma (Robinson et al, 2018; Leung et al, 2015; Paschke et al, 2013; Altinor et al; 2003).

Colchicine also suppresses the production of superoxide by neutrophils and reduces oxidative stress by decreasing the influx of calcium ions (Ca^{2+}) into neutrophils (Chia et al, 2008; Korkmaz et al, 2011). Other confirmed effects include modulation of hepatic macrophage secretion of tumor necrosis factor (TNF)α, inhibition of inflammatory cytokine

production [interleukin (IL)-1ß, interferon (IFN)γ, IL-8, IL-6], promoting dendritic cell maturation and stimulating the presentation of naive $CD4^+$lymphocyte antigens, inhibiting vascular endothelial growth factor (VEGF) and endothelial proliferation (Robinson et al, 2018; Leung et al, 2015; Slobodnick et al, 2015; Slobodnick et al, 2018; Paschke et al, 2013; Korkmaz et al, 2011).

Since 2009, colchicine has been approved by the Food and Drug Administration for use in rheumatology, immunology, cardiology, oncology, dermatology (Dasgeb et al, 2018). The use of colchicine has been shown to be beneficial for the treatment of rheumatic diseases, pericarditis, coronary heart disease, atherosclerosis, and has been attempted in various dermatological diseases, orally or topically, with variable efficacy (Karamanou et al, 2018; Robinson et al, 2018; Slobodnick et al, 2015; Slobodnick et al, 2018).

Severe aphthosis is one of the most documented indications for colchicine treatment with beneficial effects of oral colchicine being reported in case studies, case series and less often, in clinical trials (Dasgeb et al, 2018; Dalmau et al, 2007; Ruah et al, 1988; Fontes et al, 2002; Katz et al, 1994; Pakfetrat et al, 2010; Tasher et al; 2008; Butbul et al, 2016; Lynde et al, 2009).

Results and discussions

Efficacious results of colchicine treatment have been reported in several dermatologic conditions, such as chronic urticaria unresponsive to antihistamines, urticarial vasculitis, forms of cutaneous vasculitis (hypocomplementemic urticarial, leukocytoclastic, nodular, necrotic vasculitis, Henoch-Schonlein purpura), palmo-plantar pustular psoriasis (applied as a hydrophilic ointment with colchicine 1%), pyoderma gangrenosum associated or not with inflammatory bowel disease, Sweet syndrome, subcorneal pustulosis, acquired bullous epidermolysis with limited skin lesions, benign mucosal pemphigoid, Behcet's disease, actinic keratosis (applied as a hydrophilic gel with colchicine 1%) or granuloma annulare (Robinson et al, 2018; Pho et al, 2011; Jachiet et al, 2015; Goeser et al, 2014; Kontochristopoulos et al, 2004; Maillard et al, 1999; Adisen et al, 2009; Pavithran et al, 1995; Gurcan et al, 2011; Chaidemenos et al, 2011).

Less satisfactory results have been obtained for hidradenitis suppurativa, acne vulgaris, dermatitis herpetiformis, linear IgA dermatosis, scleroderma and psoriasis vulgaris (Robinson et al, 2018; van der Zee et al, 2011; Schepis et al, 1999).

Recurrent aphthous stomatitis (RAS) is a recurrent ulcerative stomatitis with an estimated prevalence between 2 and 10%, with an incompletely elucidated etiopathogenesis and, consequently, with poorly defined treatment (Altenburg et al, 2007).

In developed countries, the incidence in the general population reaches 20%, mainly affecting young adults (Bischoff et al, 2009).

The pathophysiological substrate consists of an antigenic stimulation of oral mucosal keratinocytes in predisposed individuals, followed by the secretion of proinflammatory cytokines (especially IL-2, TNFα) and the consequent expression of class I major histocompatibility complex antigens (MHC). MHC class I antigen-expressing cells become targets of cytotoxic T lymphocytes (Preeti et al, 2011; Altenburg et al, 2008).

The inflammatory process, resulting in variable epithelial necrosis depending on its histopathological site, is the consequence of an aberrant immune response, influenced by an abnormal oral flora (Leung et al, 2015).

The three modes of clinical expression of the disorder are common aphthae (a few round or oval exulcerations with an average diameter of 2-4 mm, with a gray-yellow base and a characteristic carmine-red areola, with self-limited evolution of approximately 7-10 days), herpetiform aphthae, the rarest (numerous yellowish, millimetric exulcerations, with a tendency to coalesce in erosive patches with micropolycyclicontour, evolving for approximately two weeks) and major aphthae (Sutton's ulcers or periadenitis mucosa

necrotica recurrens), the most severe clinical form (crateriform ulcers, with a diameter between 1 and 3 cm, often solitary, accompanied by satellite adenopathy, with difficult healing for 1-2 months with sometimes mutilating scars) (Peter et al, 2014; Preeti et al, 2011; Altenburg et al, 2008).

The common clinical features of these ulcerations are intense pain, location on non-keratinized areas of the oral mucosa, self-limiting character and recurrences, either spontaneous or correlated with triggering factors.

These factors may be local (e.g. oral trauma, contact hypersensitivity, sodium lauryl sulfate), nutritional deficiencies (iron, vitamin B12, folic acid), medications (angiotensin converting enzyme inhibitors, gold salts, phenobarbital, diclofenac, piroxicam), inflammatory bowel disease (gluten-sensitive enteropathy, Crohn's disease, ulcerative colitis), certain foods (tomatoes, nuts, cocoa, dairy, spices), or a hormonal context with progesterone deficiency in females. RAS is also correlated with a genetic predisposition (Leung et al, 2015; Robinson et al, 2018; Preeti et al, 2011).

Considering the clinical context of the disease, with severe episodes, only partially responsive to classic therapies, the two presented cases are part of the group of complex aphthoses (Abdulrahman et al, 2016).

The response was favorable to colchicine, starting with a dose of 1 mg/day until significant remission was obtained, and continuing with a maintenance dose of 0.5 mg/day for several months of follow-up (10 and 4 months, respectively, in our cases), recording only two mild recurrences in the patient with herpetiform aphthae and no recurrence in the patient with Sutton's ulcers (Abdulrahman et al, 2016).

Colchicine was associated with pentoxifylline in our second patient, considering literature reports of its beneficial effects in reducing the severity and frequency of aphthous ulcer episodes. Pentoxifylline inhibits the production of TNFα and reduces the migration of neutrophils, but its specific action in aphthous stomatitis is still unclear (Abdulrahman et al, 2016).

There were no side effects in any of our patients during the follow-up period.

Conclusions

In conclusion, inhibition of multiple inflammatory pathways and modulation of the innate immune response are the main attributes of colchicine exploited in the treatment of several dermatoses, including RAS.

Case studies, series of patients and clinical trials, although few, provide evidence of the efficacy of colchicine (level of evidence III) in severe aphthosis, refractory to classical therapies with topical or systemic corticosteroids, pentoxifylline, and cyclosporine.

There is no consensus on the ideal therapeutic regimen for colchicine in RAS. Therapeutic doses of 0.5-1.5 mg/day are usually free of noticeable side effects, even after 6-9 months of treatment, provided that drug interactions are avoided, and doses are adjusted in patients with hepatic or renal impairment.

The choice of treatment with colchicine in severe aphthosis should take into account the severity of the lesions, their chronic nature, the lack of therapeutic efficacy of other medications and the context of the patient morbidity.

The mechanism of action underlying the efficacy of colchicine in various dermatoses, as well as the optimal therapeutic regimen, including RAS, require further extensive research.

CHAPTER 3: RESEARCH CONTRIBUTIONS IN IMMUNE MEDIATED RHEUMATIC DISORDERS AND THEIR SYSTEMIC IMPLICATIONS

I.3.1. STATE OF THE ART

Inflammatory disease of the jaw joint and understanding how it happens is of great importance. These are divided into four main groups as synovitis, capsulitis, retrodiscite, and arthritis. Arthritis refers to the inflammation of articular surfaces of the joint. Various types of arthritis affect the temporomandibular joint (TMJ). The most common of these are osteoarthritis and polyarthritis. Polyarthritis is a systemic condition that affects all joints. Inflammation in the TMJ and tenderness in other joints manifests itself as rheumatoid arthritis (RA) characterized by pain. RA is a varied, chronic, systemic, autoimmune, and inflammatory disease that focuses on erosive symmetrical joint disease and is sometimes distinctly accompanied by extra-articular involvement. The inflammation of the synovial membranes characterizes it. This inflammation also affects the connective tissue and articular surfaces in the environment causing these surfaces to thicken and become more sensitive. TMJ involvement usually includes bilateral pain, tenderness, and swelling, as well as restriction of jaw movements. In the late phase of the disease, ankylosis is more likely to occur (Ifteni et al, 2016; Ifteni et al, 2016). RA in other joints, including the TMJ, requires medical and dental treatment (Savtekin 2018). Worldwide, the overall prevalence of RA has been reported as 0.1%–2%. It is 2–3 times more commonly seen in females and elders. Although it may be seen in all ages, the onset of the disease is mostly between the ages of 40 and 60 years (Savtekin et al, 2018).

In women, low doses of estradiol seem to induce pro-inflammatory cytokines, such as tumor necrosis factor (TNF) or interleukin (IL-) 1 beta. Although during pregnancy, estradiol doses are high, which can decrease the signalling of pro-inflammatory cytokines (Savtekin et al, 2018). Degenerative changes are seen in joints affected by RA from inflammatory cells present in synovial fluid. Macrophages, granulocytes, and plasma cells infiltrate the synovial tissues in RA (Savtekin et al, 2018). In this case, synovium becomes thicker and is called “pannus”. Pannus is the damage resulting from the invasion of the bone, cartilage, and tendons by inflammatory synovial tissue mass. The pannus grows towards the joint space and forms protruding folds. This creates pain by disrupting joint functions and causing soft tissue to stretch (Savtekin et al, 2018). Lysosomal enzymes released from granulocytes and macrophages in the synovium cause destruction and erosion in the condyle head and temporal bone. These degenerative changes may result in disruption of joint functions, fibrous and bone ankyloses, occlusal-facial deformities, and occlusal discrepancies (Savtekin et al, 2018).

Seronegative spondyloarthropathies are a family of joint disorders that classically include ankylosing spondylitis (AS), psoriatic arthritis (PsA), inflammatory bowel disease (IBD) associated arthritis, reactive arthritis (formerly Reiter syndrome; ReA), and undifferentiated SpA. Patients with seronegative spondyloarthropathy often present with inflammatory joint pain characterized by morning stiffness lasting more than one hour and improving with activity. NSAIDs may improve symptoms. There is a classic correlation between the prevalence of SpA and the prevalence of HLA-B27 gene in a given population. The strongest relationship is in AS. The worldwide prevalence of SpA is estimated to be between 0.5% to 1.9% (Sen et al, 2023). Histologic studies on synovial fluid show hypervascularity and the presence of macrophages and CD4+ and CD8+ T-cells. Interestingly, some of these same subsets of immune cells are also found in the gut mucosa. Macrophages and macrophage-driven cytokines such as TNF-alpha and IL10 are mediators of disease inflammation (Sen et al, 2023). Seronegative spondyloarthropathy is difficult to diagnose and treat. An interprofessional approach of specialty trained rehabilitation nurses,

physical therapists, and rehabilitation and rheumatology clinicians will provide the most successful management of this condition (Sen et al, 2023).

Systemic sclerosis (SSc) is a complex autoimmune rheumatic disease that is characterised by widespread skin (scleroderma) and internal organ fibrosis, immune system dysregulation, and vascular alterations (Hughes et al, 2019). SSc is a rare rheumatological condition (prevalence: ~20 per million) and is more common in females than males (~7:1) (Hughes et al, 2019). Early diagnosis is vital and key investigations include the detection of SSc-associated autoantibodies and nailfold capillaroscopic abnormalities. There is now a range of effective treatments available for many of the complications associated with the disease. Autologous haematopoietic stem cell transplantation may benefit a small subset of patients with very poor prognosis SSc. Important advances have been made in understanding the aetiopathogenesis of SSc, which is driving clinical trials of new therapeutic approaches. (Hughes et al, 2019).

A key vascular alteration in SSc is a critical imbalance between factors promoting vasoconstriction (e.g., endothelin) and vasodilation (e.g., nitric oxide) (Hughes et al, 2019). Local ischaemia (hypoxia) contributes to promote a profibrotic phenotype. Immune (both innate and adaptive) system activation is seen in SSc (Hughes et al, 2019). For example, many patients have evidence of SSc-associated antibodies and there is a rich perivascular infiltrate seen in the skin of patients with early diffuse cutaneous SSc. The close relationship between cancer and anti-RNA polymerase III antibodies further highlights the role of immunological abnormalities in SSc (Hughes et al, 2019). In these patients, there is a link between cancer-related autoantigen (i.e., mutated RNA polymerase III) recognition and an autoimmune response (Hughes et al, 2019).

There are a broad range conditions which can mimic many of the features of SSc. These include inflammatory or autoimmune diseases (e.g., eosinophilic fasciitis, graft versus host disease, nephrogenic systemic fibrosis, scleroedema, scleromyxoedema, diabetic cheiroarthropathy, amyloidosis, and carcinoid syndrome), drug-induced (e.g., aniline-contaminated rapeseed oil [toxic oil syndrome] and L-tryptophan [eosinophilia-myalgia syndrome]), and occupational exposures (e.g., epoxy resins, polyvinyl chloride, radiation fibrosis, and silica) (Hughes et al, 2019). Furthermore, a number of genetic conditions can mimic SSc, such as stiff skin syndrome and Werner's syndrome, and can occur as the result of a paraneoplastic phenomenon (Hughes et al, 2019).

I had the opportunity to observe and to study the differences between different types of rheumatological diseases, such as ankylosing spondylitis, rheumatoid arthritis, and systemic sclerosis and their impact on the patient's quality of life. There is a need of a multidisciplinary approach in preventing, diagnosing, treating and monitoring our patients. This gave me the idea of searching, analysing and understanding the immunological mechanisms.

I.3.2. SCIENTIFIC CONTRIBUTIONS

The main preoccupation that I had in this direction of research was materialized in the next papers:

Published articles

1. The Prevalence of Atopy in Biologically Treated Spondyloarthropathies: A Retrospective Study of 200 Patients, Georgiana Strugariu, Cristina Pomîrleanu, Codruta Bran, Andrei Costea, Andrei Vicovan, Diana Tatarciuc, **Irina Esanu**, Eugen Ancuta, Rodica Chirieac and Codrina Ancuta, J.Clin.Med.2022,11, 55.https://doi.org/10.3390/jcm11010055, IF=4,242

2. Immunogenicity, TNF-inhibitors levels and disease outcomes in ankylosing spondylitis: results from an observational cohort study. C. Ancuta, C. Pomirleanu, C. Belibou, R. Maxim, L. Petrariu, G. Strugariu, **I. Esanu**, R. Chirieac, E. Ancuta, Annals of Rheumatic Diseases, vol 75, supl 2, pag. 807, 2016, IF(JCR)=19.103
3. Serum Lipid Profile in Diffuse versus Limited Systemic Sclerosis Data from the SASS cohort, Ancuta C, Pomirleanu C, Iordache C, Antohe ME, Chirieac R, Ancuta E, Luchian D, **Esanu IM**, Revista de Chimie, 2018, 69(2): 403-406. IF=1.6051.
4. Temporomandibular joint involvement in rheumatoid arthritis and ankylosing spondylitis: a cross-sectional study, Iordache C, Ghiorghe CA, Antohe ME, **Esanu I**, Ancuta C. Romanian Journal of Oral Rehabilitation, 2017, 9(4): 40-46 (Web of Science Core Collection, DOAJ).

I.3.2.1. Ankylosing spondylitis

I.3.2.1.1. The prevalence of atopy in biologically treated spondyloarthropathies: a retrospective study of 200 patients

Introduction

Spondyloarthropathies (SpAs) comprise a multifactorial and heterogeneous group of chronic immuno-inflammatory rheumatic disorders primarily affecting the axial skeleton, but also presenting with peripheral (joints and enthesis) symptoms, as well as systemic damage (eye, heart, gut) (Sieper et al, 2015; Mauro et al, 2021). Owing to the high clinical variability, SpAs are currently stratified into axial SpA (axSpA) spectrum, including non-radiographic axSpAs (nr-axSpAs) and radiographic axSpAs or ankylosing spondylitis (AS), and peripheral SpAs (pSpAs), consisting of psoriatic arthritis (PsA), SpAs associated with inflammatory bowel disease, reactive arthritis, and undifferentiated conditions (Mauro et al, 2021; Rudwaleit et al, 2011). Overall, the complex and dynamic pathobiology of SpAs focuses on a T helper (Th)1/Th 2 imbalance with Th2 polarization and on the distinct intervention of Th17 cells in both articular and entheses inflammation, as well as osteoproliferative lesions labeled in AS and PsA (Mauro et al, 2021; Sharip et al, 2020; Zhu et al, 2019; Taams et al, 2018). Recognized as one important proinflammatory cytokine involved in early local and systemic inflammation of SpA, tumor necrosis factor (TNF)α remains a key player, overexpressed by Th1/macrophage axis, which is activated by interleukin (IL)-12 and IL-23 (sieper et al, 2015; Sharip et al, 2020; Zhu et al, 2019; Taams et al, 2018). On the other hand, Th2 cells are also activated in SpAs, secreting anti-inflammatory IL-4, IL-10, and IL-13 but holding the ability to modulate, or even inhibit, Th1, and to activate macrophages and lymphocytes B (Sieper et al, 2015; Mauro et al, 2021; Sharip et al, 2020). Moreover, in an attempt to restore balance, Th2 has the ability to activate the Th17 axis, both Th2 and Th17 being found in increased amounts in the serum of patients with SpAs (mauro et al, 2021; Sharip et al, 2020; Zhu et al, 2019; Taams et al, 2018). In recent decades, few papers have raised attention on the association of atopic disorders (ADs), particularly atopic dermatitis, allergic asthma, and allergic rhinitis, with immuno-inflammatory diseases including SpAs, through the common pathogenetic pathway of the IL-17/IL-23 axis with Th2 disbalance and high immunoglobulin E serum concentrations (Taams et al, 2018; Kidd et al, 2003; Chang et al, 2016; Rudwaleit et al, 2002). About 30 years ago, *Rudwaleit et al.* (1992) indicated a higher prevalence of atopy in AS patients, compared with seropositive rheumatoid arthritis (RA) (Rudwaleit et al, 2002); since then, several large studies and case reports suggested an immunological link between SpAs and atopic conditions, which is worth to be analysed in order to better understand the complexity of the immune system and find better therapeutic molecules for these patients (Karatay et al,

2013). The explanation on why patients with AS but not those with RA would have a higher rate to develop atopy resides in the Th1/Th2 paradigm which was recently updated and extended to two new players—namely, T regulatory cells (Tregs) and Th17 cells, was described (Kidd et al, 2003; Rudwaleit et al, 2002; Karatay et al, 2013). TCD4 + Treg cells are able to prevent immune responses against self-antigens and allergens by inhibiting both Th1 and Th2 cells, while Th17 and their proinflammatory IL-17 family are activated by abnormal Tregs (Orihara et al, 2008). The naturally occurring Treg (nTreg) subset contributes to prevention in autoimmune conditions, whereas the adaptive or inducible subset (iTreg), especially IL-10-producing type 1 Treg, has a well-documented role in both allergy and autoimmunity (Orihara et al, 2008). Atopic disorders share the same immune background governed by Th2 and Th17 cells and their cytokines, particularly IL-3, IL-4, IL-5, and IL-13, which actively participate in the humoral and atopic responses (Orihara et al, 2008; Rabin et al, 2008). In addition, proinflammatory Th17 cells and inducible Th2 cells able to synthesize excessive TNFα amounts are upregulated during late stages of atopic conditions resulting in symptomatic allergies (Orihara et al, 2008). Conversely, patients with asymptomatic atopy had increased number and activation of rTh2, another Th2 subpopulation, able to produce high quantities of anti-inflammatory IL-10 and to promote Th17 cells downregulation (Orihara et al, 2008). A closer look at the pathways of different atopic conditions suggests that specific mechanisms endorse their immunopathogenesis. Thus, allergic asthma is typically related to Th17 cells expressing several effector cytokines such as IL-17A and 17F, IL-22, IL-26, and granulocyte-macrophage colony-stimulating factor (GMCSF) (Wong et al, 2001); conversely, atopic dermatitis is clearly a Th2-type-driven inflammation, while allergic rhinitis is classically defined by the imbalance between Th1/Th2 response, with a predominance of Th2 and Th17 cells (Guttman-Yassky et al, 2018; Klonowska et al, 2018; Lu et al, 2021). Both SpAs and ADs are, therefore, caused by a defect in the immune system at some point during the lifetime, which promptly shifts the immune cell CD4+ pattern by activation of specific T helper subpopulations. When activation of the Th2 pattern is "on", an atopic profile is shaped, while the activation of the Th1 population and overexpression of proinflammatory cytokines (INFγ, IL-2) endorses a non-atopic profile potentially advocating for autoimmunity (Kidd et al, 2003; Rabin et al, 2008; Lu et al, 2021). This tight link between Th2, Th1, and Th17 may explain why atopic diseases, SpAs, and other immuno-inflammatory disorders can be connected (Mauro et al, 2021; Rabin et al, 2008; Lu et al, 2021). Biologic disease-modifying antirheumatic drugs (bDMARDs) addressing potent inflammatory cytokines such as TNFα and IL-17 have radically changed the outcomes of SpAs, remission, or low disease activity being achievable in routine practice in most patients (Baeten et al, 2001; Poddubnyy et al, 2018). Moreover, there are clinical studies that analyze the efficacy of biological treatment in atopic disorders (Renert-Yuval et al, 2020). While several reports emphasized that patients with AS and PsA carry a higher risk for concomitant atopic diseases, particularly allergic rhinitis and asthma (Chang et al, 2016; Rudwaleit et al, 2002), data on how drugs targeting inflammatory cytokines may influence the atopy in patients with SpA are still controversial. Indeed, in their 10-year follow-up cohort study conducted in an Asian population, *Chang et al.*, (2016) have demonstrated an increased risk of allergic diseases in AS; additionally, the authors raised the question about how DMARDs may influence the expression of atopic disorders in such patients, since biologic agents, particularly TNF inhibitors (TNFis), might affect the Th1/Th2 balance (Chang et al, 2016; Baeten et al, 2001). Impaired Th1 cytokine production in SpAs is restored by anti-TNFs, as demonstrated in 2001 by *Baeten et al.*, who evaluated the effect of TNFi on Th1 and Th2 cells in AS patients (Baeten et al, 2001). The current study aimed to evaluate the prevalence of asthma, atopic dermatitis, and allergic rhinitis in

patients with axSpA and PsA and to explore any potential association between atopic status, SpA-related parameters, and biological therapy.

Material and methods

We conducted a retrospective analysis in a cohort of 200 consecutive patients with different spondyloarthropathies (non-radiographic axial SpAs, ankylosing spondylitis, psoriatic arthritis) who are currently taking biologics (TNF and IL-17 inhibitors) to control the active disease. Their participation in the current study was based on the agreement to respond to a screening questionnaire focused on atopy, applied once between January 2020 and July 2021; moreover, eligible patients had to receive their biological treatment continuously, with no gaps in routine during the 6-month follow-up in our clinic. Participants were selected from a total of 389 patients with SpAs treated with biological therapy and who followed up between 2010 and 2021 in one academic rheumatology department in northeastern Romania. The remaining 189 patients were not eligible since they were no longer routinely monitored in our center or no longer under biological treatment due to diverse reasons (dropouts since the previous years, dropouts due to the COVID-19 pandemic, or referred to other clinics in locations more closely to their homes).

The diagnosis, classification, and management of the full spectrum of SpA were in line with expert recommendations and consensus guidelines European Alliance of Associations for Rheumatology (EULAR) and Romanian National Protocol for the treatment of axSpAs and PsA (Gossec et al, 2020; van der Hejide et al, 2016; Romanian National Protocol for the Treatment of Ankylosing Spondylitis, 2021). A brief synthesis of our local guidelines for biological therapy in axSpAs focus on several concomitant entry criteria as follows: (i) positive diagnosis of axSpA using either modified New York criteria (for AS) or ASAS criteria; (ii) active and severe disease as assessed by a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score ≥ 6 at two additional visits, at least 4 weeks apart, and Ankylosing Spondylitis Disease Activity Score (ASDAS) ≥ 2.5, together with parameters for systemic inflammation: erythrocyte sedimentation rate (ESR) > 28 mm/h and/or C reactive protein (CRP) > 3 times normal upper limit; (iii) sub-optimally controlled disease by traditional treatment meaning at least two non-steroidal anti-inflammatory drugs (NSAIDs) for at least six weeks continuously each, at maximal recommended or tolerated doses OR sulfasalazine 2–3 g/day at least 12 weeks for peripheral involvement. Similarly, patients with PsA were potential candidates for bDMARDs if they meet four concomitant criteria: (i) a definite diagnosis of PsA according to the Classification Criteria for Psoriatic Arthritis (CASPAR); (ii) severe PsA with high disease activity as definite by a Disease Activity in Psoriatic Arthritis (DAPSA) score > 28 despite conventional immunosuppressive drugs; (iii) failure to at least two conventional synthetic drugs meaning persistent active disease after 12 weeks with the maximum recommended doses, except for patients with predominantly axial PsA and those with active enthesitis and/or dactylitis, in which the use of NSAIDs in the maximum doses in the last 12 weeks is sufficient. Exclusion criteria or contraindications to biologics comprised latent or active chronic infections, severe chronic cardiac failure, history of or active neoplastic disorder. Drugs reimbursed as per local regulations include all five TNF inhibitors (TNFis) - original and biosimilars of infliximab (IFX), original and biosimilars of adalimumab (ADA), original and biosimilars of etanercept (ETA), golimumab (GLM), and certolizumab pegol (CZP) - as well as the more recently approved IL-17 inhibitor (IL-17i), secukinumab (SEK).

SpA-Related Data

For this study, we collected demographic data, disease-related parameters (a subset of SpA; type of manifestations - axial, peripheral, extra-articular; disease duration; disease activity scores), and drug-related parameters (type of bDMARDs; years of persistence on a specific drug; the number of switched bDMARDs).

Atopic Disorder Data

We applied a detailed screening questionnaire (the so-called 5Q) comprising five standardized questions specifically designed for the identification of atopy, addressed either during routine on-site visits or by phone in every enrolled case. Each question had a dichotomous answer - "yes" or "no" and patients were invited to select the answer most correctly describing their situation. In the atopic group, we included all SpA cases who positively answered "yes" to Q1 and Q2 but not those who selected "yes" for Q5 and "no" for Q1 and Q2. Moreover, Q3 and Q4 were introduced to optimize our knowledge about the potential influence of biological treatment on signs and symptoms of atopy. The screening 5Q questionnaire applied in patients with spondyloarthropathies. Item/Question YES NO Q1: Do you have knowledge about any atopic disorders such as asthma, eczema, or rhinitis diagnosed in/during your childhood? Q2: Are you currently under specific treatment for any atopic disorder such as asthma, atopic dermatitis, or rhinitis? Q3: Did you have the atopic disorder before the SpA diagnosis? Q4: Did you have the diagnosis of atopic disorder after the initiation of biological therapy for SpA? Q5: Did you have any allergic reaction to drugs, including biologics or other external chemicals? Furthermore, data on allergic asthma, allergic rhinitis, and atopic dermatitis were also collected, including time of diagnosis (during childhood (early atopy) or adulthood (late atopy)), before or after the diagnosis of SpA, key manifestations, worsening or improvement (even complete resolution) under biologics.

All patients enrolled in the study came from the north-eastern counties of Romania, having the same climate, food, or living traditions known as potential confounders in atopy. Before the initiation of biological therapy, patients were required to sign informed consent as per local procedures, while they also gave written consent for the current study. The study protocol received the approval of local ethics committees (No. 42/02.2021).

Statistical Analysis

Statistical analyses were performed using the OPENSTAT (William G. Miller, Ames, IA, USA) software; all variables had a non-parametric distribution, as demonstrated by ShapiroWilk and Lilliefors tests; Mann-Whitney and chi-squared were used in the subgroup analysis (SpA with and without atopy), and a statistically significant p-value was defined as < 0.05). On the other hand, in the PsA cohort, patients were almost equally distributed among genders (46% female), and 91% were non-smokers ($p < 0.05$).

Results

Disease-Related Parameters Detailed subgroup analysis was further performed. The axSpA patients are distributed as follows: 131 (65%) AS cases, including those with AS associated with inflammatory bowel disease (6 cases, 3%), and 12 (6%) cases with non-radiographic axSpA; more than half (84 cases, 58.7%) presented also peripheral manifestations. Among PsA patients, 41 (72%) had symmetrical polyarticular involvement, 14 (24.5%) axial involvement, and 2 (3.5%) arthritis mutilans. We reported a statistically significant difference ($p < 0.00001$) when comparing axial and peripheral involvement between subgroups.

Biologic Drugs

Incontestably, the majority of cases in our cohort were on TNF inhibitors (mainly adalimumab, etanercept, infliximab, but also the newer agents, e.g., golimumab and certolizumab) since this class of biologics was, for many years, the only reimbursed by our health insurance. Thus, 157 (78.5%) patients were prescribed a TNFi, 40.1% adalimumab, 35.03% etanercept, 9.5% certolizumab, 8.2% infliximab, and only 5.7% golimumab; among them, 89 (56.7%) were still on their first biological agent, while 68 (43.3%) changed between two and five TNF inhibitors. Only 43 patients (21.5%) were taking IL17 inhibitors, 21 cases (48.8%) 150 mg monthly secukinumab (18 AS, 3 PsA) and 22 (51.2%) patients the full dose

of 300 mg (18 PsA, 4 AS). In addition, we reported a mean duration since the first administration of biological drugs of 6.6 years (1–18 years) for TNFi and only 0.44 years (1–3 years) for IL17i, explained by the relatively recent approval of anti-IL-17 agents for the treatment of active SpA and local reimbursement starting from 2017.

Prevalence of Atopy

We stratified patients in atopic and non-atopic SpA based on their answers on the 5Q screening questionnaire; the atopic group comprised all patients who answered "yes" in Q1 and Q2, meaning that they had or still have atopy (current atopy) diagnosed and followed up by a specialist. Overall, atopic disorders were reported in up to 51 (25.5%) patients of our cohort; among them, 37 cases (72.5%) presented with a form of atopy in their medical history before SpA was diagnosed, while 14 patients (27.4%) were diagnosed with atopy during the evolution of SpA.

Conversely, patients who positively answered to Q5 (meaning that they have an allergy to drugs or other external chemical substances and no other atopic disorders) were excluded from the atopic group. Overall, 24.4% of axSpA and 28% of PsA patients have atopy; there is no statistically significant difference between the proportion of patients with atopy in subgroup analysis, as calculated by using the chi-squared test ($p = 0.598$).

We failed to identify any significant difference in demographic variables between atopic and non-atopic cohorts ($p > 0.05$, chi-squared test). Furthermore, there were no statistically significant differences ($p > 0.05$) in axial and peripheral involvement between atopic and non-atopic patients: 73% axial and 62% peripheral manifestations in the atopic cohort, as compared with 96% axial and 56% peripheral involvement in non-atopic SpA, respectively.

However, we reported significant differences only in concomitant inflammatory bowel disease, 7 out of 12 patients with intestinal involvement being among atopic SpA ($p = 0.007$); intestinal involvement could be an independent factor for atopy, and further studies are required to assess the link between the intestinal microbiome and atopy mechanisms in patients with SpAs.

Conversely, ocular involvement was not evaluated since axSpAs a priori develop more acute anterior uveitis than PsA and we enrolled predominantly axSpA patients in our cohort.

Additionally, we could not demonstrate any significant difference between mean disease history, delay from onset to diagnosis, and the number of years under biological treatment in atopic SpA, compared with non-atopic SpA ($p > 0.05$).

One potential explanation relies on eligibility criteria for biologics according to local recommendations comprising long-term history, SpAs incompletely controlled by classical medication, and multiple negative prognostic factors.

Furthermore, SpAs with and without atopy have the same global persistence on biologics; mean exposure to TNF biologics was 6.9 years (median of 8 years, range between 1 and 16 years) for atopic SpAs and 6.5 in non-atopic SpAs ($p > 0.05$), and the same mean exposure of 0.4 years (1–3 years) for atopic and non-atopic SpAs ($p > 0.05$) for IL-17 inhibitors.

In total, 67 (46%) non-atopic SpA patients were classified as non-responders in more than two anti-TNF agents, and 79 patients (54%) in this group required only one TNF inhibitor to control disease activity (Figure 3.1-A).

We were also interested in evaluating the number of switching required to control SpA activity—namely, 20 patients (39.2%) in the atopic cohort switched at least one TNF inhibitor and 12 patients (23.5%) needed more than three anti-TNF molecules, while 25.5% (13 patients) only one anti-TNF in order to attain sustained remission or, at least, low disease activity (Figure 3.1-B).

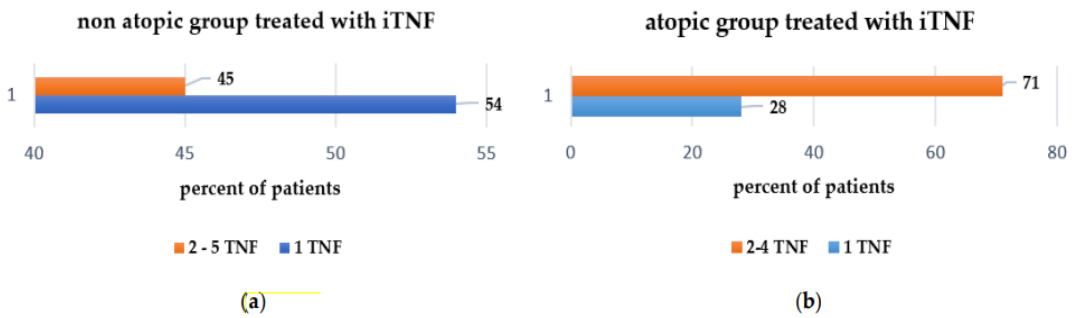


Fig. 3.1. Number of TNF inhibitors used to treat SpAs patients: **(A)** non-atopic; **(B)** atopic cohort

Details on the number of TNF inhibitors administered for each cohort (atopic and non-atopic) are provided in Figure 3.2 and Table 3.I clearly shows that significantly more patients in the non-atopic cohort (54%) are controlled by their first TNF inhibitor, compared with those in the atopic cohort (28%).

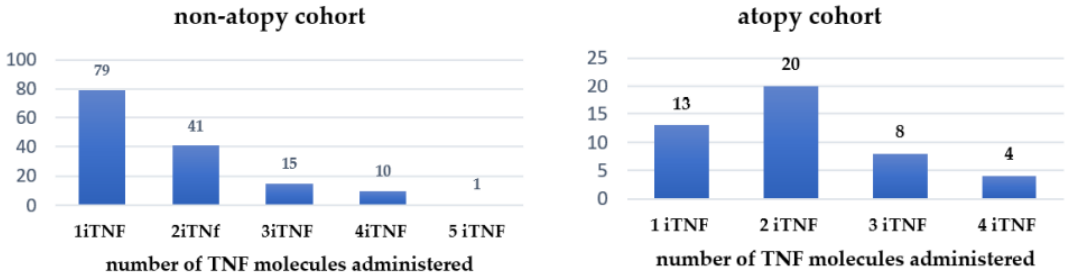


Fig. 3.2. Number of TNF inhibitors used to treat SpA patients: (a) non-atopic; (b) atopic cohort

Number of Patients Treated with TNF Inhibitors and Number of TNF Needed						
	1 TNFi	**2 TNFi**	**3 TNFi**	**4 TNFi**	**5 TNFi**	**Number of Patients**
Atopy cohort	13 (22.04) (3.71)	20 (14.61) (1.98)	8 (5.51) (1.12)	4 (3.35) (0.12)	1 (0.48) (0.57)	46
Non atopy cohort	79 (69.96) (1.17)	41 (46.39) (0.63)	15 (17.49) (0.35)	10 (10.65) (0.04)	1 (1.52) (0.18)	146

The chi-squared statistic is 9.8745. The p-value is 0.042596. The result is significant at $p < 0.05$. Calculated with https://www.socscistatistics.com/tests/chisquare (accessed on 12 December 2021).

Table 3.I. Distribution of patients according to the number of TNF inhibitors switched

SpA patients with atopy required 1.92 TNF inhibitors (median od 2, range 1–4 molecules) versus 1.7 for SpA patients without atopy ($p > 0.05$). Although statistically non-significant, there is a numeric difference between patients taking 300 mg monthly of secukinumab (13 atopic patients versus 28 non-atopic patients).

Further studies are necessary with comparable cohorts of patients (number, disease subtype, exposure period) to evaluate the influence of IL-17 inhibitors (drug, dose) on atopic symptoms. Patients with atopy also needed a higher dose of secukinumab in 25.5%, as shown in Table 3.II.

Table 3.II. Characteristics of atopic and non-atopic patients

Table 3. Characteristics of atopic and non-atopic patients.

Parameter	Atopic Group 51 (25.5%)	Non-Atopic Group 149 (74.5%)	*p*-Value Significant $p^* < 0.05$
Gender, *n* (%) Expected total/chi-square Male Female	 36 (70.6) (37.74) (0.08) 15 (29.4) (13.26) (0.23)	 112 (75.2) (110.26) (0.03) 37 (24.8) (38.74) (0.08)	 0.51 *
Living style, *n* (%) Expected total/chi-square Urban Rural	 28 (55) (28.3) (0) 23 (45) (22.7) (0)	 83 (56) (82.7) (0) 66 (44) (66.31) (0)	 0.92 *
Age (years), *n* (%) Expected total/chi-square <20 21–40 41–60 >60 Mean age	 0 (0) (1.29) (0.07) 13 (25.4) (14.49) (0.15) 29 (56.8) (23.54) (1.27) 9 (17.6) (12.68) (1.07) 48.8	 4 (2.6) (3.71) (0.02) 43 (28.8) (41.51) (0.05) 62 (41.6) (67.46) (0.44) 40 (26.8) (36.32) (0.37) 48.4	 0.775064 0.643731 0.059035 0.18739 1
Smoking status, *n* (%) Expected total/chi-square Smoker Nonsmoker	 8 (15.6) (11.48) (1.05) 43 (84.4) (39.52) (0.31)	 37 (23.8) (33.52) (0.36) 0.17 112 (76.2) (115.48) (0.1)	
Skeletal involvement, *n* (%) Expected total/chi-square Axial Peripheral	 35 (73) (39.62) (0.54) 30 (62.5) (25.38) (0.84)	 143 (96) (138.38) (0.15) 84 (56) (88.62) (0.24)	 0.17 0.09
Intestinal inflammatory symptoms, (*n*) % Expected total/chi-square	7 (14.5) (3.06) (5.07)	5 (3.3) (8.94) (1.74)	 0.007
Duration of disease since onset ** Mean ± SD Median (Range)	16.11 ± 10.06 13 (37)	15.94 ± 9.37 14 (43)	 0.99 *
Years from onset to diagnosis ** Mean ± SD Median (Range)	3.17 ± 5.92 1 (33)	2.74 ± 5.34 1 (33)	 0.80 *
Years of diagnosis SPA ** Mean ± SD Median (Range)	12.98 ± 9.40 10 (36)	13.16 ± 8.85 11 (44)	 0.87 *
Years of TNF inhibitors ** Mean ± SD Median (Range)	 6.93 ± 4.09 8 (15)	 6.53 ± 4.00 6 (16)	 0.86 *
Years of IL-17 inhibitors ** Mean ± SD Median (Range)	 0.48 ± 0.81 0 (3)	 0.46 ± 0.86 0 (4)	 0.98 *
Number of TNF inhibitors ** Mean ± SD Median (Min, Max)	 1.92 ± 1.00 2 (1, 4)	 1.7 ± 1.00 2 (1, 5)	 0.98 *
Patients on 300 mg IL-17inhibitors (*n*) % expected total/chi-square	 13 (25.5) (10.46) (0.62)	 28 (18.8) (30.54) (0.21)	 0.30 *

* *p* calculated with chi-squared calculator online: https://www.socscistatistics.com/tests/chisquare (accessed on 12 December 2021); ** parameters expressed by the mean, standard deviation (SD) and median with range calculated with OPENSTAT programs, Shapiro–Wilk, Lilliefors, and Mann–Whitney tests.

Discussion

The current study was specifically designed to assess the prevalence of atopic disorders among patients diagnosed with SpAs (either axSpA or PsA) and ongoing biological therapy and to identify potential correlations between atopy and demographic data, disease, and bDMARD-related parameters in a cohort of 200 patients. Despite emerging data on cytokine patterns in AS and various atopic conditions such as asthma, atopic dermatitis and allergic rhinitis, the association between atopy and immune-mediated rheumatic conditions is still controversial. Indeed, different studies have underpinned the mutual inhibition of Th1 and Th2 axes, suggesting that Th1 and Th2 polarized diseases exclude each other, e.g., atopy (Th2 polarization) provides protection for the development of rheumatoid arthritis (Th1 polarization) and vice versa but associates with ankylosing spondylitis (Th2 polarization) (Kidd et al, 2003; Rudwaleit et al, 2002; Rabin et al, 2008; van Roon et al, 2002). *Rudwaleit et al.,* (2002) have analyzed the risk of atopy in Caucasian patients with rheumatoid arthritis or ankylosing spondylitis; overall, atopic disorders were more prevalent in AS (24.6%), compared with seropositive RA (13.1%), but non-significantly increased, compared with controls (20.7%) (Rudwaleit et al, 2002).

We have also shown comparable data—one in four patients with SpA and up to one-third of those with PsA have a concomitant atopic disorder in our monocentric retrospective cohort; these results are also similar to those reported in the literature. When analyzing different atopic conditions separately, the prevalence of asthma, allergic rhinitis, and atopic dermatitis were highest in AS, intermediate in controls, and lowest in the RA population (Rudwaleit et al, 2002). Moreover, patients with AS have a 1.31 times greater risk of developing asthma, 1.46 times higher risk for allergic rhinitis, and 1.22 times for atopic dermatitis, compared with the general population, according to the results published by *Chang et al.* (2015), in a 10-year follow-up, population-based study in Taiwan (Chang et al, 2016). Data are confirmed by Shen et al. (2015), who documented the increased risk of allergic asthma in AS under various pathogenic medications, regardless of age and sex, also in an Asian cohort (Shen et al, 2015). A recent study suggests that atopy is not commonly associated with psoriasis vulgaris, supporting the concept that Th2-mediated atopy protects against certain Th-1-mediated autoimmune pathologies such as psoriasis, in which we discussed the intervention of the proinflammatory cytokine axes TNF, IFN type 1, and IL-17 (Guttman-Yassky et al, 2018; Hasseini et al, 2019). We demonstrated a higher prevalence of allergic rhinitis (54%), compared with atopic dermatitis (35%) and allergic asthma (20%) in our cohort, similar to data reported in other studies that showed a higher incidence of rhinitis in SpA patients [10]. A closer examination of the potential correlation between atopy and gender, age, disease duration, and severity revealed that all patients in our atopic cohort were mainly male (70%), older than non-atopic patients, aged more than 35 years old (40–60 years), and more than half featuring an urban lifestyle. Conversely, *Chang et al.* (2018) demonstrated a significantly higher incidence of AD in patients with AS younger than 20 years old, and a trend of a higher incidence rate of allergic diseases in different age groups; however, the authors point to the small case numbers in each age group, suggesting that it may not be powered to detect the difference. Moreover, they recommended monitoring related signs and symptoms of atopic dermatitis in younger patients with AS (Chang et al, 2016). Overall, it seems that the prevalence of atopic disorders in our cohort is comparable irrespective of the activity and management, suggesting that the biological therapy does not change the frequency of atopy. In other words, concomitant atopic conditions remained unchanged even after the initiation of bDMARDs in patients with a well-defined atopic status. Moreover, we could not identify any correlations between persistent atopic disease and negative prognostic factors for SpA, theoretically related to specific criteria for the access to biologics in our country that result in a homogeneous population. Only one factor

could potentially influence the clinical significance of atopy in our patients, and this is related to the concomitant inflammatory bowel disease—namely, 14.5% of cases in the atopic subgroup were associated with intestinal inflammation, three times higher than the non-atopic patients. To the best of our knowledge, this is the first analysis of atopic disorders among severe active SpA taking biologics in a Romanian population. Despite discrepancies between subgroups (number of patients, rate of TNFi users compared with IL-17i users, exposure to TNFi versus IL17i), one major advantage of the study is the relatively homogeneous population in terms of SpA activity, as biologics are prescribed according to well-established local recommendations. One potential limitation of the current study is that the majority of patients in our cohort had a long history of their SpAs (a mean of 15 years), with a mean exposure to anti-TNF agents for more than six years; since the persistence on IL-17 inhibitors was under four years, correlations between the type of biologics and frequency of atopic disorders are not feasible. Furthermore, we could not correctly value the role of IL-17 inhibitors on atopic conditions. However, we showed that atopic patients required more frequent switches to different biologic drugs in order to achieve the therapeutic target (1.92 TNF inhibitors compared with 1.7 in non-atopic subjects), as well as higher doses of secukinumab (300 mg monthly) in 25.5% of patients, compared with 18.8% non-atopic cases. Although statistically non-significant ($p > 0.05$), we suggest that atopic disorders may be related to difficult-totreat SpAs. Further studies should be performed in larger cohorts of patients and with comparable exposure, in order to correctly evaluate the effectiveness of different classes of biologics on atopic conditions.

Conclusions

We successfully confirmed that atopic disorders, particularly rhinitis, atopic dermatitis, and asthma may develop in patients diagnosed with spondyloarthropathies who are taking biological therapy. Additionally, irrespective of their spondyloarthropathy, it seems that atopic patients require more frequent switching among biologics to control their active disease. Further studies are necessary to investigate if the presence of concomitant atopy could prioritize the selection of appropriate biologic drugs, in order to achieve fast and lasting disease control in patients with SpAs.

I.3.2.1.2. Immunogenicity, TNF-inhibitors levels and disease outcomes in ankylosing spondylitis: results from an observational cohort study

Introduction

Up to one third of spondylarthritis (SpA) fail to respond to anti-TNF agents or experience drug toxicity leading to treatment withdrawal. Part of the treatment failure can be explained by the development of anti-drug antibodies (ADA). The objective of this study was to evaluate the relation between immunogenicity, drug levels and clinical efficacy of TNF inhibitors (TNF-i) in ankylosing spondylitis (AS) (Arstikyte et al, 2015; Hoxha et al, 2015).

Material and methods

We performed a prospective observational study in a cohort of 47 consecutive AS patients receiving adalimumab (ADL) (13; 27.7%), infliximab (IFX) (13; 27.7%) or etanercept (ETN) (21; 44.7%).

Disease activity (BASDAI, ASDAS), outcomes and adverse events were evaluated at baseline and study visit, while serum TNF-i and ADA levels collected as a single-point data in both bio-naïve (37, 78.7%) and bio-experimented patients. Serum drug levels were considered positive for IFX if >0.035 μg/mL, for ADL >0.024 μg/mL and for ETN >0.035 μg/mL, while the cut-off value for the ADA positivity to IFX was established at 5AU/ml,

for ADL at 10AU/mL and ETN at 142 AU/mL (ELISA, Progenika). Statistical analysis was performed using SSPS version 19.0, $p<0.05$.

Results

At baseline mean BASDAI was 7.69 and mean ASDAS-CRP 3.50, with no difference in disease activity between patients who did or did not later develop ADA ($p<0.05$). 37 (78.7%) AS were BASDAI responders at study visit (BASDAI 1.16, ASDAS-CRP 1.73).

ADA were detected in 8/47 (17%) and were more frequent in patients treated with ADL (5 cases; 38.5%) vs IFX (3 cases; 23.1%); no with ETN. Both ADL and IFX levels were significantly higher for ADA negative than for ADA positive patients (ADL: 3.92μg/mL vs 0.02μg/mL, $p<0.01$; IFX: 1.82μg/mL vs 0.03 μg/mL, $p<0.01$).

A significant association between clinical activity (ASDAS) and immunogenicity (ADA status) was reported: patients who had developed ADA had higher disease activity (3.10 vs 1.73, $p<0.01$), and more patients were classified as being in a high or very high disease activity status.

Furthermore, a relation between clinical improvement (change in ASDAS) and immunogenicity was reported: ADA-positive AS achieved worse clinical response than ADA-negative cases, with a significant association between ADL respectively IFX levels and ASDAS ($p<0.05$).

Discussion

TNF inhibitors (ADL, IFX) have revolutionized the treatment of AS by targeting the overactive immune response. However, the interplay between immunogenicity, drug levels, and clinical efficacy of TNF- α in AS patients is a complex and crucial area of study. Adalimumab is a fully human monoclonal antibody that selectively binds to TNF-α, while infliximab is a chimeric monoclonal antibody that neutralizes both soluble and membrane-bound TNF-α (Arstikyte et al, 2015; Hoxha et al, 2015). ADAs can neutralize the therapeutic effect of TNF-α by forming immune complexes. Long-term exposure to TNF-α inhibitors can lead to the development of neutralizing ADAs, reducing the sustained efficacy of the drugs. This highlights the need for continued monitoring, potential adjustments, and a proactive approach to managing immunogenicity to ensure sustained clinical activity (Arstikyte et al, 2015; Hoxha et al, 2015).

The association between clinical activity and immunogenicity of ADL and IFX in the treatment of ankylosing spondylitis is a critical aspect that informs treatment decisions. The presence of ADAs can compromise their clinical activity, emphasizing the importance of monitoring, individualized treatment strategies, and efforts to mitigate immunogenicity's impact on AS patients' well-being.

Conclusions

ADL and IFX levels are commonly influenced by ADA-positivity, and related to clinical response in AS, suggesting that therapeutic drug monitoring should be investigated as a possible tool to optimise treatment such patients.

I.3.2.2. Systemic sclerosis

I.3.2.2.1. Serum lipid profile in diffuse vs limited systemic sclerosis data from the SASS cohort

Introduction

Data about lipoprotein changes and their link with cardiovascular disease and atherosclerosis in systemic sclerosis (SSc) are still challenging. We aimed to evaluate serum lipid profile of patients with SSc and to identify potential relation with different disease specific characteristics (clinical, serological, inflammatory tests) in a cross-sectional study.

Standard assessments comprised SSc-related parameters (disease subtype, clinical spectrum, immunological tests) and lipid metabolism (total cholesterol and fractions, triglycerides). Impaired lipid profile (low serum HDL- and high LDL-cholesterol, increased serum triglycerides, slightly modification in total cholesterol level) significantly correlated with diffuse SSc, activity (EUSTAR) and severity (MEDSGER), as well as seropositivity for specific antibodies (anti-centromere and anti-topoisomerase 1). The dyslipidemic profile might represent a pathobiological pathway for atherosclerosis in SSc (Magda et al, 2015; Pagkopoulau et al, 2017; Au et al, 2011).

Material and methods

We performed a cross-sectional observational study in consecutive SSc patients (fulfilling either 1980 ACR diagnostic criteria or new 2013 ACR/EULAR classification criteria) enrolled in the SSAS (Early Accelerated Atherosclerosis in Systemic Sclerosis) cohort, attending at least once the outpatient Rheumatology Department in the 162 EUSTAR Center of Iasi, Romania). Standard assessments collected during the routine monitoring visit in our clinic included:

- general data about SSc, such as disease subtype (limited or diffuse cutaneous SSc; 1988 LeRoy classification), clinical spectrum, autoantibodies (total antinuclear antibodies
- ANA, anti-topoisomerase-1, anticentromere) and complement levels (C3 and C4 fractions), disease activity (EUSTAR) and severity (MEDSGER), treatment with synthetic anti-rheumatic drugs;
- lipid profile, comprising serum total cholesterol (TC), low- and high-density lipoprotein cholesterol fractions (LDLC, HDL-C), triglycerides (TG) as well as the atherogenic plasma index (AI).

Lipids were determined as fasting levels using our local laboratory, and lipid levels of risk were classified in accordance with latest NCEP and AHA/ACC recommendations, as follows: plasma total cholesterol more than 200 mg/dL, HDL-cholesterol more than 40 mg/ dL, LDL-cholesterol more than 130 mg/dL, while triglycerides more than 200 mg/dL, respectively.

None of enrolled patients was taking concomitant lipid lowering drugs (statins), corticosteroids, beta-blockers or diuretic, given the possible influence on lipid concentrations.

Moreover, we excluded those with comorbidities known to potentially alter lipid profile, such as diabetes, coronary artery disease, hypertension, thyroid disease.

All participants have signed a written informed consent before their enrollment and the study received Ethics Committee approval. Statistical analysis was done in IBM SPSS-19 software, $p<0.05$, with a subgroup analysis based on skin extent (diffuse, limited skin involvement) and serology profile (seropositive or seronegative patients). Multivariable regression analysis was done to evaluate association between lipid metabolism parameters and different SSc settings.

Results

SSc-related parameters A total of 86 SSc were enrolled in the study, with a slight predominance of those presenting with the limited SSc (lcSSc) (48 cases, 55.81%) vs. diffuse SSc (dcSSc) subtype. Patients were mainly women (76 cases, 88.37%), in their fourth decade, with a mean age 45.3 (23-65) years and mean disease duration of 67 (16-127) months. We described a wide clinical spectrum in our SSc, including both visceral and non-visceral involvement as follows: all patients presented Raynaud phenomenon, with digital pitting scars in 38 cases (44.18%), active digital ulcers in 25 cases (29.07%) and critical ischemia in only three cases (3.48%) at the time of the study; both nonerosive and erosive arthritis were described in 60 cases (69.76%), with consistent hand disability in up to one third of patients (30 cases, 34.88%) and subsequent altered quality of life (as appreciated by

using a Health Assessment Questionnaire, mean score of 1.9 points). Only 16 (18.60%) of our SSc had patent myositis, with moderate impact on daily activities, while significantly more patients had subclinical muscle involvement. Interstitial lung disease was present in 71 (82.55%) patients, while pulmonary arterial hypertension only in few cases (15 individuals, 17.44%). Cardiac arrhythmias were reported in 31 cases (36.04%), being symptomatic in about one third of them. In addition, very frequent gastrointestinal involvement (dysphagia and reflux due to esophageal dysmotility) was reported in 63 cases (73.25%).

However, no scleroderma renal crisis or other types of renal involvement were found in the enrolled SSc. Positive inflammatory syndrome (erythrocyte sedimentation rate, ESR; C reactive protein, CRP) was noticed in up to half of cases, with mild to moderate increase particularly in ESR, while CRP concentrations were abnormal in 30 cases (34.88%).

Specific SSc-autoantibodies were detected as follows: anti-centromere antibody (ACA) positivity in 36 cases (41.86%), all diagnosed with lcSSc subtype, while antitopoisomerase-1 specificity (anti-topo1 or anti-Scl70 antibodies) in 32 cases (37.20%), all being classified as dcSSc subtype; furthermore, the majority of cases presented with ANA positivity (70 cases, 81.39%) and one third complement consumption (as defined by decreased C3 levels). SSc-related parameters are summarized in Table 3.III and lab assessments in Table 3.IV.

Regarding the medication, the majority of patients were on methotrexate (doses ranging between 10 and 20 mg once weekly, given for skin, articular and muscle complains), only four of them on cyclophosphamide (pulse-therapy monthly as they presented with severe symptomatic interstitial lung fibrosis) and five on cyclosporine A (skin, cardiac, erosive arthritis refractory to conventional immunosuppressants); all of them were taking calcium-channel blockers and pentoxifylline for their vascular disease, and those with esophageal signs and symptoms on proton pomp inhibitors and prokinetic drugs.

Lipid profile in SSc A complex assessment of the serum lipid metabolism parameters was performed as per protocol in all SSc patients, supporting the following data: mean total cholesterol level of 182.85 (120-300) mg/dL, mean HDL cholesterol of 45.23 (35-70) mg/dL, mean LDL-cholesterol of 136 (125-200) mg/dL, while mean serum triglycerides were detected at a serum concentration of 157.2 (115-245) mg/dL (Table 3.V).

Table 3.III. General SSc data in studied patients

Parameters	Values
Demographics	
Age (years)	45.3 (23-65)
Gender (%)	83.87% women
Disease-related	
Disease duration (months)	67 (16-127)
Disease subset	
• dcSSc (%)	44.19
• lcSSc (%)	55.81
Disease activity (MEDSGER)	1.63 (0-2)
Disease severity	4.4 (3-6)
Skin score (modified RODNAN, mRSS)	17 (6-38)
Interstitial lung disease (%)	82.55
Pulmonary hypertension (%)	17.44
Raynaud phenomenon (%)	100
Active digital ulcers (%)	29.07
Digital pitting scars (%)	44.18
Critical digital ischemia (%)	3.48
Myositis (%)	12.90
Arthritis (non-erosive, erosive) (%)	69.76
Cardiac arrhythmias (%)	36.04
Esophageal involvement (%)	73.25%

Table 3.IV. Serologic and biochemical analysis in SSc

Lab parameters	
Antibodies positivity	
Anti-centromere, ACA (%)	22.58
Anti-topo1(%)	58.06
ANA (%)	87.09
Inflammatory syndrome	
CRP (mg/dL)	6.2 (1-14)
ESR (mm/hour)	29 (15-64)
Lipid profile	
Cholesterol (mg/dL)	182.85 (120-300)
HDL-cholesterol (mg/dL)	45.23 (35-70)
LDL-cholesterol (mg/dL)	136 (125-200)
Triglycerides (mg/dL)	157.2 (115-245)

Table 3.V. Lipid profile in dcSSc *vs.* lcSSc

Lipid profile	Diffuse SSc	Limited SSc	P
Low HDL-cholesterol (%)	71.05	37.5	<0.05
High LDL-cholesterol (%)	57.89	31.25	<0.05
High triglycerides (%)	69.52	39.58	<0.05
Total cholesterol (%)	71.05	39.58	<0.05

Overall, we reported the aberrant lipid profile in more than half of studied SSc, the so-called scleroderma- lipid pattern comprising low HDL-cholesterol and high LDL-cholesterol fractions, with slightly increased in serum triglycerides, without a significant modification in total cholesterol levels.

We also completed an individual subgroup assessment of lipid profile in both disease subsets (dcSSc and lcSSc);we registered consistent differences among dcSSc and lcSSc patients ($p<0.05$), meaning significant much more lipid abnormalities in diffuse vs. limited disease.

Patients with dcSSc showed lower serum HDL-cholesterol levels (71.05%) and higher LDL-cholesterol(57.89%) concentrations as compared to lcSSc (37.5% for HDL-cholesterol fraction; 31.25% for LDL-cholesterol,respectively) ($p<0.05$). Furthermore, significant more cases in the dcSSC subgroup had higher triglycerides (69.52 vs. 39.58%,$p<0.05$) as well as lower cholesterol levels (71.05% vs.39.58%, $p<0.05$) as compared to lcSSc, particularly in patients fulfilling the criteria for CREST syndrome (anacronym for Calcinosis, Raynaud, Esophageal dysmotility, Sclerodactyly, Telangiectasia). A detailed analysis of fasting lipids revealed the following abnormalities: mean total cholesterol was significantly lower for dcSSc than for lcSSc patients (127.65 mg/dL vs.190.01mg/dL, $p<0.05$); mean HDL-cholesterol concentrations were lower for dcSSc patients than for lcSSc (43.5mg/dL vs. 61.4 mg/dL, $p<0.05$); the same trend was detected for the LDL-cholesterol fraction (128.6 mg/dL vs. 167.5 mg/dL) as well as for the triglycerides (112.78mg/DL vs. 200 mg/dL).

Correlation between lipid metabolism and SSc-related parameters

We further evaluated potential relations between lipid metabolism parameters and different disease related (clinical, serologic) characteristics. We identified significant link between lipid modifications and the extent of skin involvement (high RODNAN skin score positively correlates with lipid anomalies, $p<0.05$), disease duration (longer history of SSc

promotes more dyslipidemic changes, $p<0.05$), SSc activity (higher EUSTAR score associates with more significant lipid modifications, $p<0.05$) and SSc severity (higher MEDSGER severity scores, higher lipid abnormalities, $p<0.05$). Moreover, patients with seropositivity for either ACA or anti-topo-1 antibodies had significant more dyslipidemic profile than those without serologic abnormalities (ACA negative or anti-topo-1 negative subgroup) ($p<0.05$).

We assumed that patients with SSc are at risk to develop impaired lipid profile, meaning low HDL-cholesterol and high LDL-cholesterol fractions, high serum triglycerides, compromised total cholesterol levels, potentially involved in cardio-vascular disease and atherosclerosis.

Discussions

Previously, we have already published data on impaired lipid pattern in different SSc settings and scenarios (patients included in the SASS cohort) (Zeng et al, 2012; Ancuta et al, 2017; Ancuta et al, 2016; Lippi et al, 2006; Borba et al, 2005); however, available data on total cholesterol and its fractions, low-density and high-density lipoprotein cholesterol, triglycerides and lipoproteins (A, B) in patients diagnosed with SSc, with special emphasis on different disease subsets remains still controversial (Ancuta et al, 2017; Ancuta et al, 2016; Lippi et al, 2006; Borba et al, 2005). In addition, it is widely accepted that immune mediated rheumatic conditions such as lupus, rheumatoid arthritis, even spondyloarthropathies, are characterized by abnormal lipid profiles (the pretended lipid paradox), both traditional and non-traditional (disease-related) cardio-vascular risk factors accounting for high burden of cardiovascular disease and atherosclerosis (Oreska et al, 2017; Magda et al, 2015; Nordin et al, 2010; Belibou et al, 2012; Toms et al, 2011).

Both inflammation and immune abnormalities are linked with proatherogenic lipoprotein profile, comprising high LDL-cholesterol and triglycerides, together with low HDL-cholesterol and impaired total cholesterol, promoting early accelerated atherosclerosis in autoimmune rheumatic or non-rheumatic pathologies (Magda et al, 2015; Nordin et al, 2013; Dimitrulas et al, 2014; Zeng et al, 2012; Ancuta et al, 2017; Ancuta et al, 2016; Lippi et al, 2006; Borba et al, 2005; Gheorghe et al, 2017).

In the current study we reported defective serum lipids in a cohort of consecutive SSc patients, particularly in those classified as diffuse SSc subtype (elevated LDL-cholesterol, decreased HDL-cholesterol, high triglycerides and total cholesterol). Our data partially support data from literature investigating the potential contribution of lipoprotein abnormalities to vascular complications in SSc (Missala et al, 2012; Toms et al, 2011; Ngian et al, 20012; Psarras et al, 2017; Dimitrulas et al, 2014; Zeng et al, 2012; Ancuta et al, 2017; Ancuta et al, 2016; Lippi et al, 2006; Borba et al, 2005; Gheorghe et al, 2017).

Certain authors inconsistently reported altered total cholesterol and its fractions (low-density and high-density lipoprotein cholesterol), triglycerides and lipoproteins (LpA and B). Thus, total cholesterol, low density lipoproteins, high density lipoproteins, triglycerides, atherogenic index of plasma were either altered correlating with certain clinical (PAH) and immunological (anti-centromere, antitopo1 antibodies) parameters (Oreska et al, 2017; Lippi et al, 2006), or presented no adverse alterations (Oreska et al, 2017; Borba et al, 2005). A closer look to lipid changes in our SSc patients highlighted that abnormal lipids were related to high RODNAN skin score, longer disease duration, anti-topo1 positivity, SSc activity and severity (EUSTAR, MEDSGER severity scale). Finally, further studies are mandatory in order to clearly define the robust role of lipid abnormalities as traditional cardio-vascular risk factors and potential relations with disease-related parameters in patients with SSc.

Conclusions

Patients with SSc are at risk to develop abnormal lipid profile (with low serum HDL-cholesterol and high LDL-cholesterol levels, high triglycerides, and total cholesterol),

particularly those with a diffuse cutaneous SSc, those with active and severe disease, and specific autoantibodies.

Furthermore, the dyslipidemic profile might represent one of the pathobiological pathways for atherosclerosis in SSc.

I.3.2.3. Temporomandibular joint in rheumatic conditions

I.3.2.3.1. Temporomandibular joint involvement in rheumatoid arthritis and ankylosing spondylitis: a cross-sectional study

Introduction

The aim of our study was to identify potential relations between temporomandibular joint (TMJ) pathology (signs and symptoms) and disease activity, disability and impairment of quality of life in patients with inflammatory rheumatic conditions. We performed a cross-sectional observational study in a cohort of consecutive patients with inflammatory rheumatic disorders (IRD) and TMJ-related arthritis, attending at least once the outpatient rheumatology department between 2005 and 2007. 152 patients with rheumatoid arthritis (RA) and 55 with ankylosing spondylitis (AS) met the eligibility criteria (IRD with TMJ involvement at the time of examination) and were recruited for this study; supplementary, 33 healthy controls also featured signs and symptoms related to TMJ involvement and qualified to be included in the study. TMJ complaints were recorded by a regular questionnaire examining the following items: spontaneous muscle pain, muscle pain during use of the jaw, articular pain, difficulty in opening the mouth. In all cases we evaluated disease activity and disability according to internationally validated instruments specifically designed for each disorder or with a common destination. TMJ involvement is commonly reported in patients with RA and AS account for high levels of disability and impaired health-related quality of life. TMJ arthritis significantly correlates with disease activity and disability, not only in RA but also in AS, requiring a complex management.

Chronic inflammatory rheumatic disorders (IRD) such as rheumatoid arthritis (RA), ankylosis spondylitis (AS) and other spondylarthropathies (SpA) are broadly characterized by a significant economic and illness burden, mainly related to disease activity, severity, as well as disability and impaired quality of life (Amandeep et al, 2015; Cordeiro et al, 2016; Sodhi et al, 2015). Despite early diagnosis, aggressive therapy according to treat-to-target strategy, close monitoring in line with specific guidelines and recommendations, up to 40% of patients are not controlled with specific therapies (synthetic remissive or biological anti-rheumatic drugs) (Amandeep et al, 2015; Cordeiro et al, 2016). Temporomandibular disorders refer to us as various clinical pathologies concerning the jaw muscles and temporomandibular joint (TMJ), among them IRD being highly susceptible to develop signs and symptoms related to TMJ arthritis (Amandeep et al, 2015; Cordeiro et al, 2016; Ifteni et al, 2016; Ifteni et al, 2016). Thus, it is estimated that more than half of the patients with IRD, particularly rheumatoid arthritis but also ankylosing spondylitis, psoriatic arthritis juvenile idiopathic arthritis have clinical evidence of either bilateral or unilateral TMJ involvement (Ifteni et al, 2016; Tataru et al, 2016; Kallenberg et al, 2013). Furthermore, the substrates of TMJ pathology encompass for the same inflammatory as well as destructive events as in other joints affected by specific rheumatic diseases (Davidson et al, 1975; Antohe et al, 2016; Kallenberg et al, 2013); the clinical picture includes a wide spectrum of manifestations such as joint and muscle pain, TMJ sounds, muscle spasm, swelling, stiffness, opening derangements and bruxism, restricted movements, with major impact on the quality of life (Amandeep et al, 2015; Cordeiro et al, 2016; Antohe et al, 2016; Goupille et al, 1993).

Predictably, there is a strong association between TMJ arthritis and disease activity scores, severity as well as disability, with a substantial contribution of disease duration in compromising oral health in patients with rheumatoid arthritis or psoriatic arthritis (Davidson et al, 1975; Helenius, 2005; Antohe et al, 2016). A complex, multidisciplinary approach of TMJ pathology during IRD is mandatory, requiring an early diagnosis and systemic plus local management in order to decrease the burden of structural and functional TMJ damage (Amandeep et al, 2015; Pallak et al, 2013; Ramos-Remus et al, 1997). The aim of our study was to identify potential relations between TMJ pathology (signs and symptoms) and disease activity, disability and impairment of quality of life in patients with inflammatory rheumatic conditions.

Material and methods

We performed a cross-sectional observational study in a cohort of consecutive patients with inflammatory rheumatic disorders (IRD) and TMJ-related arthritis, attending at least once the outpatient rheumatology department from January 2005 to July 2007. 152 patients with rheumatoid arthritis (RA; ACR 1987 diagnostic criteria) and 55 with ankylosing spondylitis (AS; 1984 modified New York criteria) met the eligibility criteria (IRD with TMJ involvement at the time of examination) and were recruited for this study; supplementary, 33 healthy controls also featured signs and symptoms related to TMJ involvement and were qualified to be included in the study.

TMJ complaints were recorded by using a regular questionnaire examining the following items: spontaneous muscle pain (visual analogue scale 0-10), muscle pain during use of the jaw (chewing) (present/absent), articular pain (present/absent), difficulty in opening the mouth (present/absent). In all cases we evaluated disease activity and disability according to internationally validated instruments specifically designed for each disorder or with a common destination.

Thus, for RA we calculated disease activity by DAS28-ESR (Disease Activity Score on 28 evaluable joints using erythrocyte sedimentation rate) and disability by HAQ (Health Assessment Questionnaire). DAS28 is a combined index that has been developed to measure the activity in RA and calculated using several individual parameters such as swollen and tender joints, patient's global disease assessment and the erythrocyte sedimentation rate. Validated cut-offs for DAS28-ESR clearly split the RA in high disease activity (HDA; DAS28-ESR> 5.1), moderate disease activity (MDA; DAS28- ESR between 3.2 and 5.1), low disease activity LDA; DAS28-ESR between 2.6 and 3.2) and remission (REM) if DAS28-ESR < 2.6. AS activity was assessed by the BASDAI score (Bath Ankylosing Spondylitis Disease Activity Index), while disability was quantified by BASFI (Bath Ankylosing Spondylitis functional index).

The study protocol was approved by local Ethics Committee and all patients have signed a written informed consent before study initiation. Statistical analysis was done in SAS 4.3 program (descriptive and analytical statistics including chi-squared, Pearson's correlation, Breakdown one way ANOVA in all the groups described above), $p<0,5$. 55.14±12.81 years (range between 20 and 83); about 79% had moderate to severe radiological damage; 94.74% of cases had more than 6 tender joints, while 84.87% more than 6 swollen joints; in addition, according the articular index Ritchie (including TMJ) known to define the aggressiveness of the disease, almost all patients (98%) presented with more than 10 affected joints.

Results

Finally, disability is widely described in AR related either to inflammation (early stages of the disease) or tissue damage (advanced stages), with consistent influence on the quality of life. According to the Steinbroker's classification, our patients had mild functional

impairment in about 11.84% cases and moderate disability in 47.379% (inflammation), while 40.79% had severe joint damage with consecutive limited function (Table 3.VI).

One third of our RA (31.58%) was in HDA at the moment of their rheumatologic appointment, more than half (66.45%) showed MDA, while only a very small percentage of patients (1.97%) fall into REM (Table 3.VII).

We arbitrarily considered mild disability if HAQ < 1, moderate for HAQ between 1 and 2.5, while severe disability corresponds to a HAQ between 2.5 and 3. 38.82 % of our patients presented with significantly impaired quality of life (HAQ more than 2.5) and 46.71% with moderate disability as shown in Table 3.VII.

Table 3.VI. Individual RA parameters

Stage I		Stage II		Stage III		Stage IV	
N	%	n	%	N	%	n	%
10	6.58	47	30.92	73	48.03	22	14.47

Swollen joint count				Tender joint count			
< 6		> 6		< 6		> 6	
N	%	n	%	N	%	n	%
23	15.13	139	84.87	8	5.26	144	94.74

< 10 joints (Ritchie index)		>10 joints (Ritchie index)	
N	%	N	%
3	1.97	149	98.03

Steinbroker functional capacity stage I		Steinbroker functional capacity Stage II		Steinbroker functional capacity Stage III & IV	
N	%	n	%	n	%
18	11.84	72	47.37	62	40.79

Table 3.VII. DAS-28-ESR and HAQ in RA patients

High disease activity DAS28>5.1		Moderate disease activity (DAS28=3.2-5.1)		Remission DAS28<2.6	
N	%	n	%	N	%
48	31.58	101	66.45	3	1.97

Mild impairment (HAQ<1)		Moderate impairment (HAQ =1-2.5)		Severe impairment (HAQ = 2.5-3)	
n	%	n	%	n	%
22	14.47	71	46.71	59	38.82

Data about TMJ involvement in RA are shown in tables below (Table 3.VIII).

Pain at digital palpation of the jaw muscles and TMJ are widely considered related to both inflammation and articular destruction; 52.63% of RA described pain at pretragus region palpation, while 34.13% at external auditory canal level. Pain was commonly described in the masseter muscle (53.29%) as well as medial pterygoid muscle (40.13%), and only in 29.61% was related to temporal muscles. Muscle spasm was frequently identified in the temporal muscle (57.24%), 28.95% medial pterygoid and 11.84% of masseter muscle. Further analysis revealed a significant association of TMJ pain with the number of involved joints (> 4 swollen joints; $\chi2=4.78$, $p=0.029$; and > 10 tender joints, $\chi2=5.87$, $p=0.015$), disability (HAQ >2.5; $\chi2=12.97$, $p=0.0003$) as well as Ritchie index ($\chi2=4.96$, $p=0.02$). Moreover, the absence of pain in TMJ was typically reported in those RA patients in remission according to DAS28-ESR (<2.6) (5.17% vs. 0%, p = 0.027).

Table 3.VIII. Pain and muscle spasm in RA patients at the TMJ level

TMJ pain					
Present			Absent		
N	%		N	%	
94	61.84		58	18.16	
Pretragus region pain					
Present			Absent		
N	%		N	%	
80	52.63		72	47.37	
Auditory external canal pain					
Present			Absent		
N	%		N	%	
53	34.87		99	65.13	
Muscle pain					
Masseter		Temporal		Medial pterygoid	
N	%	N	%	n	%
81	53.29	45	29.61	61	40.13
Muscle spasm					
Masseter		Temporal		Medial pterygoid	
N	%	N	%	n	%
18	11.84	87	57.24	44	28.95

AS and TMJ involvement Spondyloarthritis (SpA) represent a heterogeneous group of chronic inflammatory conditions, with significant morbidity and disability related to both articular and extra-articular features. AS, the disease-prototype for the concept of SpA, is defined by early inflammation as well as late ossification events. We enrolled 55 patients with AS and TMJ-related arthritis. 80% were males, with mean age of 44.36±12.04 years (ranging between 20 and 80 years) and mean disease duration of 15.49±8.68 years (ranging from 2 to 44 years). All patients had axial disease, and more than half (31 cases, 56.36%) developed peripheral arthritis. Up to 55% cases presented severe sacroiliac joint involvement (ankylosis, stage IV), and about one third (34.55%) significant narrowing of the articular space (stage III sacroiliitis). AS activity and severity were evaluated according to the internationally validated indexes BASDAI and BASFI, as summarized in Table 3.IX.

Table 3.IX. BASDAI and BASFI scores in AS patients

BASDAI (0-10)			BASFI (0-100)		
	n	%		n	%
Normal	1	1.82	Normal	4	7.27
Mild disease activity (3-4)	21	38.18	Mild disability (30-40)	7	12.73
Moderate disease activity (5-7)	25	45.45	Moderate disability (50-70)	27	49.09
High disease activity (8-10)	8	14.55	High disability (80-100)	17	30.91

About half of cases (45.45%) had moderate disease activity, with BASDAI values of 5 or 6, 38.18% were in low disease activity, while 14.55 in high activity. In addition, BASFI covered moderate (49.09%) and high (30.9%) functional disability in a substantial proportion of patients (Figure 3.3).

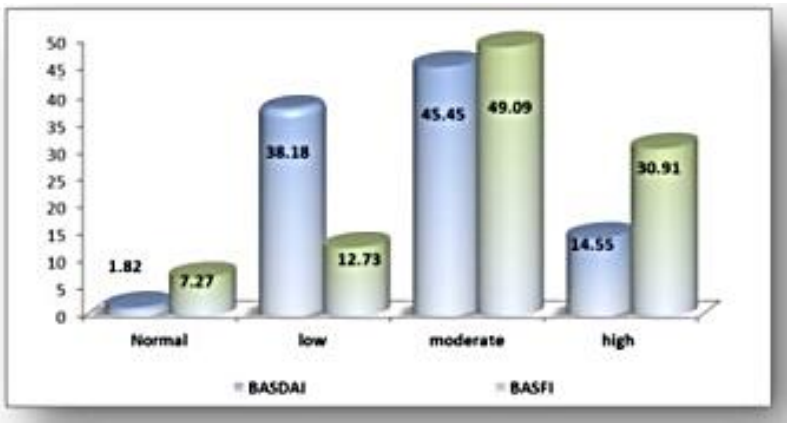


Fig. 3.3. Disease functional activity in BASDAI and BASFI

Signs and symptoms of TMJ arthritis in AS are further described. 45.45% cases had unilateral TMJ involvement. The majority of AS patients (89.09%) had TMJ pain, 67.27% felt pain at palpation of the external autitory canal, 52.73% at palpation of pretragus region, 49.43% at mastication; finally, 32.73% AS featured also TMJ stiffness. Extended analysis showed further associations between TMJ signs and symptoms and AS activity and functional scores (Table 3.X).

Table 3.X. BASDAI, BASFI and TMJ signs and symptoms

BASDAI	Pain at mastication	3.50	.02
	Pain at palpation of the external auditory canal	4.37	.008
	Pretragus region pain	3.23	.03
	Pain when closing mouth	3.24	.03
	Noises when opening mouth	2.87	.045
	Pain in the temporal muscle	6.67	.0007
	Spasm of the temporal muscle	2.91	.04
BASFI	Pain at mastication	6.19	.001
	Pain at palpation of the external auditory canal	3.41	.02
	Noises when opening mouth	3.17	.03
	Pain in the temporal muscle	3.70	.02

TMJ in controls 33 subjects belonging to the control group were also analysed; 11 male (33.33%) and 22 female (66.67%), with mean age of 55.15±10.41 years (between 32 and 78). We included in the control group patients attending the rheumatology department for one of the following rheumatic conditions: sciatica (45.45%), acute low back pain (21.21%), cruralgia (12.12%), as well as chronic low back pain and fractures (forearm). TMJ signs and symptoms were detected unilaterally in 21.22% cases; the majority of patients recruited in the control group (87.88%) presented pain at palpation of the pretragus region, 40% at the external auditory canal. All cases had TMJ stiffness. Muscle pain was also commonly registered at palpation of the masseter muscle (75.76%) and medial pterygoid muscle (42.42%), while muscle spasm involved frequently the temporal muscle (81.82%)

Comparative analysis of RA, AS and controls

A complex clinical as well as lab assessment of the TMJ, dento-periodontal and specific evaluation of the RA and AS was carried out and materialized our research database. Pain, a cardinal symptom of musculoskeletal pathology with precise connotations in IRD was systematically analysed highlighting TMJ involvement in our patients. We studied the potential impact of TMJ related arthritis on disease activity and disability in patients with RA or AS. Patients with inflammatory rheumatic disorders and TMJ involvement experience significant levels of activity and functional impairment, with subsequent influence on health-related quality of life. Therefore, we found that about 90% of RA subjects and near 80% of

those diagnosed with AS had moderate to high level of disability. Functional capacity in AR, AS and controls in our study Mild disability (level I) was described significantly more frequent in the control group (p =0.006, 0.008); moderate health impairment (level II) was found significantly more frequently in AR patients (p=0.02, 0.001), and higher degree of disability (level III) was also significantly more common in AR (p=0.045). In order to establish significant variations in the TMJ damage index according to the factors included in the study, we proceeded to ANOVA one way analysis; table below summarizes variables with statistical significance (Snedecor F coefficient, p<0,05).

Discussion

Bilateral pain, tenderness, swelling, and limitation of jaw movements are distinct characteristics of in RA of the TMJ. There may be no radiological findings in the early stages, but in later stages, the condylar articular surface is degenerated, the joint gap is narrowed, and anterior open bite can be seen. In children, this degeneration can lead to growth retardation and facial deformity in the mandible. In adults, it varies from hardening of the joint to occlusal facial deformities. There is a high likelihood of ankyloses in all patients (Ifteni et al, 2016; Savtekin et al, 2018). In 1874, Garrod reported RA in TMJ involvement for the first time. The TMJ may have unilateral involvement as well as symmetric involvement (Savtekin et al, 2018).The most common clinical symptom is deep preauricular pain during function. Joints are sensitive to palpation, and joint stiffness is present in the morning. There is also a decrease in click, creep, and bite strength (Savtekin et al, 2018). Moen et al. (Moen et al, 2005) reported pain and dysfunction from TMJ in 77% of RA patients with the most common deformity being anterior open bite. Lin et al. (Lin et al, 2007) reported that, in their study of 56 patients with RA, fibrous and bone ankyloses accompanied anterior open bite in 3 patients. Patients with TMJ-RA have clinical findings such as joint sounds, myalgia-related musculature, and limited mandibular movement. TMJ condylar destruction is observed in early RA disease and can be investigated 6 months after the first diagnosis. Also, after long-term follow-up, radiographic findings, such as erosion, flattening, and resorption of the condyle was most commonly observed (Bessa-Nogueira et al, 2008). Initial symptoms of oropharyngeal dysphagia (OD) can be seen as a consequence of joint deformation. A decrease in the mandibular movement, including fatigue, pain, masticatory difficulties, and improvement in chewing and swallowing duration has a link to several oral preliminary and oral stage OD signs and symptoms, which leads to weight loss. These signs and symptoms of OD can decline the quality of life in the RA population (Gilheaney et al, 2017; Savtekin et al, 2018).

Conclusions

TMJ involvement is commonly reported in patients with rheumatoid arthritis and ankylosing spondylitis and account for high levels of disability and impaired health-related quality of life. Pain (articular, jam muscle), TMJ stiffness, restricted motions, TMJ sounds as well as opening derangements remain major clinical signs and symptoms of TMJ arthritis as a result of both inflammatory and destructive damage of the articular and soft tissue lesions. TMJ arthritis significantly correlates with disease activity and disability not only in rheumatoid arthritis but also in ankylosing spondylitis, requiring a complex management.

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CHAPTER 4: RESEARCH CONTRIBUTIONS IN THE FIELD OF ONCOLOGY

I.4.1. STATE OF THE ART

Contemporary cancer care is, of necessity, highly multidisciplinary, involving medical specialists from medical, surgical and radiation oncology, pathology, radiology, palliative care, rehabilitation medicine, and many other disciplines. As cancer treatment decisions are increasingly influenced by the results of sophisticated molecular testing, new specialists, such as molecular pathologists, genomics experts, and bioinformaticians, are being added to the cancer care team. Due to the severity of the underlying illness, the necessary treatments for cancer are often associated with significant morbidity and reduced patient quality of life. (Berghmans et al, 2020; Chang et al, 2021).

The most common cancer diagnosed in both sexes is lung cancer (11.6% of the total cases), followed by breast cancer in women (11.6%) and prostate cancer in men (7.1%) (Bray et al, 2018).

Colorectal cancer (CRC) is third in terms of recognition (6.1%) and second in terms of mortality (9.2%). It is estimated that by the year 2035, the total number of deaths from rectal and colon cancer will increase by 60% and 71.5%, respectively (Sawicki et al, 2021).

These figures may differ from country to country depending on the degree of economic development. Therefore, the disease is widely recognized as a marker of the country's socioeconomic development respectively (Sawicki et al, 2021).The increase in morbidity is also influenced by lifestyle, body fatness and dietary patterns respectively (Sawicki et al, 2021). There is convincing evidence that physical activity has a protective effect. The risk of developing the disease is increased by more frequent red and processed meat and alcohol drinks respectively (Sawicki et al, 2021). The progress of civilization and economic development, apart from improving socioeconomic conditions, also causes a change in dietary patterns, referred to as the westernization of the lifestyle. This means higher consumption of animal fats, processed meats, refined grains or sweets, a low supply of dietary fibers, fruits vegetables and low physical activity. The occurrence of overweight or obesity is often the result of such a lifestyle respectively (Sawicki et al, 2021). Overweight and obesity are associated with an increased risk of many civilization diseases. Visceral obesity has been reported to adversely affect the prognosis of CRC in men respectively (Sawicki et al, 2021). About a quarter of a contributor to genetic predisposition. The development time of CRC usually lasts from several to several years; therefore, it is very important to diagnose it early in developing the disease. Based on follow-up examinations and nutrition prevention based on a balanced diet, secondary prevention is also important respectively (Sawicki et al, 2021). In recent years, the global burden of CRC will increase by 60%, to over 2.2 million new cases and 1.1 million deaths by 2030. Such a significant increase will be the result of economic development, an economic transformation consisting in the transition from lowto-medium-HDI nations and generational changes in developed countries. Many research studies emphasize that this increase is also the result of environmental changes, such as a more sedentary lifestyle, abnormal bony weight (obesity), consumption of highly processed food, alcohol, red meat consumption and an increase in overall life expectancy respectively (Sawicki et al, 2021).

Osteosarcoma (OS) is a malignant tumor that originates in the mesenchymal tissue (which constitute spindle-shaped stromal cells that can produce bone-like tissues), and it accounts for 20% of all cases of primary malignant bone tumors in the world (Zhao et al, 2021). In fact, it is the most common type of primary malignant bone tumour among adolescent patients (Zhao et al, 2021). The incidence of OS is common in the metaphysis of long tubular bones (such as the proximal humerus, the distal femur, and the proximal tibia), but rare in the spine, pelvis, and sacrum areas (Zhao et al, 2021). The majority of patients with OS present with only a single lesion (Zhao et al, 2021). Clinically, the onset of the

disease is characterized mainly by local pain and swelling, and occasionally by joint dysfunction. A few patients have also been treated for pathological fractures. The symptoms of growth pain and trauma are confounding, but the degree of malignancy is high (Zhao et al, 2021). Notably, nearly 10–20% of the patients are affected by measurable metastatic disease before actual onset, the most common site being the lungs (85%), followed by the bones (8–10%) and, occasionally, the lymph nodes. The remaining 80–90% of the patients can be considered to possess subclinical or micrometastases, which cannot be detected accurately by using the presently available diagnostic methods (Zhao et al, 2021). The presence of metastatic disease is a clear indication of poor prognosis of OS (Zhao et al, 2021). OS is a rare sarcoma that has the histological findings of osteoid production in association with malignant mesenchymal cells (Zhao et al, 2021). OS is the third most common cancer in adolescence, with only lymphomas and brain tumors being more prevalent, and with an annual incidence of 5.6 cases per million children under the age of 15 (Zhao et al, 2021). Peak incidence is in the second decade of life Before the age of five, OS is rare OS arises sporadically, with few cases associated with known inherited defects in cell cycle regulation, but about 70% of tumor specimens demonstrating a chromosomal abnormality. These commonly involve mutations in tumor-suppressor genes or in DNA helicases (Zhao et al, 2021; Misaghi et al, 2018). The World Health Organization's histologic classification of bone tumors divides OS into central, intramedullary, and surface tumors, with a number of subtypes under each group (Misaghi et al, 2018).

Conventional treatment for OS consists of a combination of neoadjuvant and adjuvant chemotherapy, and surgery (Misaghi et al, 2018). Prior to the use of chemotherapy, there was less than a 20% survival rate in high-grade conventional osteosarcoma even with surgical amputation, indicating the presence of micrometastases (typically pulmonary) prior to surgery (Misaghi et al, 2018). The low grade can typically be treated with excision alone and chemotherapy is avoided if final pathology confirms low grade (Misaghi et al, 2018).

In the last decades, the number of patients with neoplastic pathology has increased, so I have turned my attention in the detailed study of at least 3 types of neoplasia, among which I mention colorectal cancer, bone cancer, but also cancer in the gynaecological sphere.

I.4.2. SCIENTIFIC CONTRIBUTIONS

The main preoccupation that I had in this direction of research has been realized by publishing the following articles:

Published articles

1. Effect of TAT-DOX-PEG irradiated gold nanoparticles conjugates on human osteosarcoma cells, Lupusoru R, Pricop DA, Uritu CM, Arvinte A, Coroaba A, **Esanu I**, Zaltariov MV, Silion M, Stefanescu C, Pinteala M. *Scientific Reports* 2020; 10:659. https://doi.org/10.1038/s41598-020-63245-8 IF=3,998
2. Chemotherapy and other chemical factors influencing the quality of life of patients with hereditary nonpolyposis colorectal cancer, Chirica VA, Boanca M, Matei M, Postolica R, Chelaru L, **Esanu IM**, Sanduleac L, Porumb V, Coman EA, Azoicai D. *Appl. Sci.* 2020; 10(18):6585. https://doi.org/10.3390/app10186585 IF=2,474
3. The Overexpression of Folate Receptors in Gynecologic Oncology - a Diagnostic and Therapeutic Approach, Popovici R, Cărăuleanu A, Lazar TI, Petroaie A, Pangal A, Grigore M, **Esanu I**. *Rev. Chim. (Bucharest)* 2019; 70(12):4532-4536. https://doi.org/10.37358/RC.19.12.7789. IF=1,755

I.4.2.1. Bone oncology

I.4.2.1.1. Effect of TAT-DOX-PEG irradiated gold nanoparticles conjugates on human osteosarcoma cells

Introduction

The presence of gold nanomaterials (AuNPs) in biomedicine and particularly in antitumor therapy remains a topic of wide debate, as evidenced by the tremendous amount of scientific works on this issue in recent years (Singh et al, 2018; Vines et al, 2019; Jahangirian et al, 2019). An impressive number of research studies have straightened their efforts toward the use of AuNPs in enhancing the efficiency of cancer treatment, due to their ease production and chemical functionalization of their surface (Conde et al, 2014; Tiwari et al, 2011).

The present work aimed to conduct insightful studies of biological effects starting from previous outcomes involving polymer-coated gold nanoparticles subjected to green light irradiation. According to a previous research (Andries et al, 2016) concerning fungal cultures treated with AuNPs suspensions, the first line of defends, expressed by antioxidant enzymes, was quantified by analysing the activity of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) content.

The greatest stimulation of CAT and SOD was induced after incubation of cellulolytic fungi cultures with gold nanoparticles irradiated with green light, as compared to similar nanoparticles irradiated with other wavelengths. Since an increased level of ROS has been generated after incubation of fungal cells in the presence of green light irradiated AuNPs, similar irradiated particles are expected to produce comparable effects on human cells, inhibiting the growth of tumor cells.

The nanoparticles were designed to be irradiated just after their stabilization in sodium citrate, for the reason that at this stage the metal atoms could modify their oxidation state with an influence on organic layer conformation (Park et al, 2018).

Material and methods

Gold(III) chloride trihydrate (HAuCl4·3H2O, Mw=393.83 g/mol), sodium hydroxide (NaOH), sodium citrate (Na3C6H5O7·2H2O), polyethylenglycol diglycidyl ether (PEG500, Mn ~ 500 g/mol) and doxorubicin (DOX, Mw = 579.98 g/mol) were purchased from Sigma Aldrich. Tiol PEG amine hydrochloride (HS-PEG2000- NH2·HCl, Mn ~ 2000 g/mol) was obtained from JenKen Technology USA, and cysteine-terminated TAT peptide (TAT-cys, Mw = 1315 g/mol) of 95% purity (GRKKKRRQRC) from ChemPeptide Limited company.

Structural characterization. Fourier-transform infrared spectroscopy (FTIR). FTIR spectra were obtained in transmission mode using a Bruker Vertex instrument, model 70. The samples were prepared by depositing the nanoparticle suspension on KBr pellets which were then subjected to a drying process (using a UV lamp) before recording the spectra. The spectra ranged from 4000 to 400 cm−1 with a resolution of 2 cm−1 . X-ray Photoelectron Spectroscopy (XPS). XPS data were achieved on an Axis NOVA instrument (Kratos Analytical, Manchester, United Kingdom), using AlKα (1486.6 eV) as X-ray source, with 20 mA current and 15 kV voltages (300W), under a base pressure of 10−8 ÷10−9Torr in the sample compartment. The incident monochromatic X-ray beam was focused on a 0.7mm ×0.3mm XPS area of the sample surface.

The high-resolution spectra for all the elements of interest were the average of five scans acquired using a pass energy of 20 eV and a step size of 0.1 eV. The binding energy of the C 1 s peak, normalized at 284.6 eV, has been established as reference value for all binding energies. XPS data fitting was accomplished using the ESCApe sofware, by applying Gaussian-Lorentzian mixed function.

Results

Following the synthesis protocol described in Materials and Methods section and illustrated by Figure 4.1, a 2.5 mM suspension of AuNPs was obtained, comprising particles of approximately 17nm in diameter (16.83±0.25nm) with long-term stability (over 6 months, determined by macroscopic evaluation and confirmed by DLS and UV-Vis spectroscopy). The enhanced stability of the AuNPs suspension is mainly due to the use of a high pH value during AuNPs formation process in the presence of sodium citrate (Li et al, 2011). It is well known that at high pH, the citrate is fully deprotonated, creating a high abundance of negative charges, inducing repulsions between nearby gold nanoparticles and as a result no aggregation can be obtained.

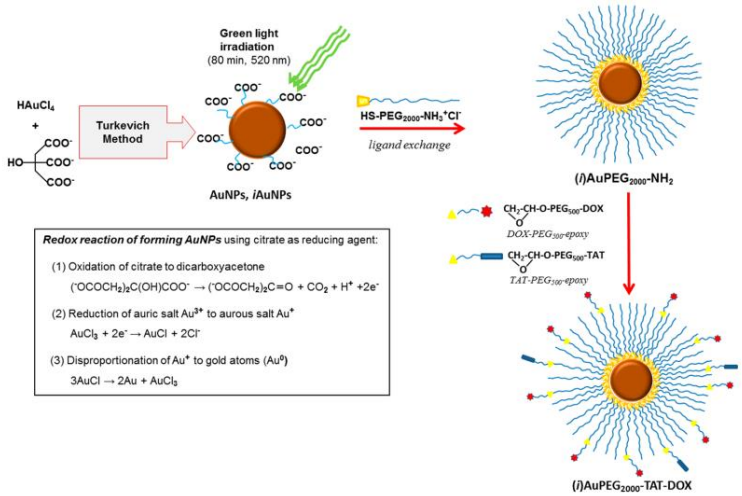

Fig. 4.1. The main steps of AuNPs synthesis followed by surface PEGylation, and conjugation with doxorubicin and TAT peptide

Discussions

Gold nanoparticles are feasible to be developed as versatile nontoxic carriers for drug release as long as they are able to be conjugated with different molecules, including chemotherapeutics, antibodies, peptides, ligands, and other structures which are likely to promote a great capacity to penetrate the tumor site, resulting in a predominant accumulation of bioactive agent in the tumor region (Peng et al, 2019; Sztandera et al, 2019). On the other hand, the passive anticancer effect based on the accumulation strategy of AuNPs at the tumor site is limited by the inherent heterogeneities of tumor vasculature (Jain et al, 2012). It was shown that nanoparticle concentration in the target tissue is influenced by renal clearance rate, and also by activation of immune system mechanisms such as opsonization or nonspecific particle phagocytosis, fulfilled by the reticuloendothelial system (RES).

Different strategies for surface functionalization of AuNPs using a wide range of ligands have been done to overcome these limitations. Polyethylene glycol (PEG) is the polymer known as the most popular material for surface modification in various types of nanoparticulate drugs or gene delivery systems (Ardeleanu et al, 2018; Clima et al, 2015; Dascalu et al, 2017; Lazarus et al, 2016; Uritu et al, 2015). The coverage of the conjugates with PEG moieties plays a major role in improving solubility and stability in aqueous media of the carriers, prolonging the circulation time in the blood stream, bypassing immune recognition due to steric hindrance mechanism (Bunker et al, 2012; Cho et al, 2010; Suk et al, 2016). A significant number of works have been concerned about the structure of citrate adlayers on gold nanoparticles, elucidating the binding modes of carboxylate to metallic surface as a consequence of reaction parameters (Al-Johani et al, 2017; MacLeod et al, 2019; Polte et al, 2010). Starting from the classical Turkevich method, the synthesis was enhanced by adding sodium hydroxide to the reaction medium, thus obtaining a stable colloidal

solution having a concentration ten times higher in gold than in a traditional protocol (from 0.25 to 2.5 mM). Further polymer coating with PEG, as it can be seen later in this paper, is logically influenced by the oxidation states of gold atoms and also the binding feature between citrate and particle surface (Martynyuk et al, 2016; Wang et al, 2010). The PEGylation using a heterobifunctional polyether derivative was designed to displace the citrate ligand due to formation of stronger Au-S coordinative bonds, although Au-COO− from citrate linkages may also be present, explaining the sporadic occurrence of amine terminal end of PEG in gold surface vicinity, based on electrostatic interaction between -COO− and -NH3 + (Dinkel et al, 2016; Hong et al, 2005). A different approach of antitumor therapy was considered in the framework of current research, by which doxorubicin (DOX), one of the most investigated chemotherapeutic agents, was covalently bound to the surface of polymer coated gold nanoparticles (Rivankar et al, 2014). The coupling was envisioned through an oxirane bifunctional linker, able to bind both DOX and PEG due to the presence of a primary amino group (Young et al, 2006). A second important issue was to achieve an efficient penetrability into the tumor cells by the drug loaded particles. Tus, a small number of TAT-peptide grafted onto the polymeric shell has been found as a convenient strategy, with acknowledged results in cellular internalization of non-self-structures and with a highly potential of tumor targeting, as reported in literature (Borelli et al, 2018; Regberb et al, 2012; Wang et al, 2008). FTIR and XPS spectroscopy proved to be of crucial importance in establishing the structure of the final products, with a focus on the differences produced by irradiation. The structure elucidation was completed by morphological and dimensional data performed by TEM and DLS, which reveal uniform entities not exceeding 20 nm in diameter, as disclosed later in this work. The positive ζ potential of the nanoparticles, due to the protonated amino groups onto the polymer coating surface, provide the advantage of being opposite to the cell surface charging, thus facilitating their transfer through cell membranes33. Biological tests have revealed that our drug-free carriers (AuPEG2000-NH2 and iAuPEG2000-NH2, comprising only the metallic core coated with PEG) do not exhibit cytotoxic effects on normal human dermal fibroblasts and human osteosarcoma. Moreover, the loaded carriers with doxorubicin were more efficient when TAT peptide was attached to the system.

Conclusions

The present research aimed to design an antitumor agent based on gold nanoparticles with superior efficacy in the context of minimal drug loading, in order to avoid adverse effects on healthy tissues, and ensuring at the same time an acceptable colloidal stability. The gold nanoparticles stabilized by sodium citrate were irradiated in visible light at 520nm before being coated with polyethylene glycol. Both macroscopic examination and surface analysis have shown that the irradiation enhanced the particle stability even at higher concentrations, keeping a narrow dimensional distribution. Advanced structural characterization techniques highlighted several differences between irradiated products against the non-irradiated ones, which can explain the colloidal behaviour of the particles, their stability, and also the auspicious antitumor activity, as reported by literature data. The resulting structures may have the potential to avoid systemic toxicity and side effects on healthy tissues when used as drug carriers, if considered the viability of normal versus malignant cells under the influence of unloaded nanoparticles. Another reason for such favourable results, with a great potential for deepened research, is the covalent binding of doxorubicin, leading to a new molecule that may act differently, in a synergistic way with the free drug adsorbed into the coating. Under the conditions of present study, the main achievement is that a similar cytotoxic effect has been obtained on malignant HOS cells when using only 3% doxorubicin (loaded into nanoparticles) as compared to pure drug treated cells.

I.4.2.2. Gastrointestinal oncology

I.4.2.2.1. Chemotherapy and other chemical factors influencing the quality of life of patients with hereditary nonpolyposis colorectal cancer

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide, with over 1.8 million new cases per year, of which approximately 500,000 new cases are in Europe (Gheorghe et al, 2020). CRC can be hereditary in 3% of patients, with an onset at younger ages (Argillander et al, 2018; Chirica et al, 2015). The increasing incidence of CRC emphasized the studying of quality of life (QoL) in patients undergoing oncological and surgical therapies. The health deterioration due to cancer along with the consequences regarding psychological, physical or social functioning changes, all of them influencing the QoL (Utescu et al, 2019). Several studies highlighted the impact on QoL of CRC protocols in patients, immediately after chemoradiation and in the long run (Marventano et al, 2013). The QoL for cancer patients is associated with a greater number of factors (Hinganu et al, 2019), such as: socio-demographic characteristics; health-related factors; factors related to cancer and surgical procedures; lifestyle (Fodor et al, 2019); other factors (Poroch et al, 2015; Poroch et al, 2014). The stage and location of CRC at the time of diagnosis is important in quantifying QoL, as they determine the symptoms, treatments and duration of therapy (ferlay et al, 2018).

The stage I patients showed a positive progressive tendency in the QoL score. The stage IV patients had a negative score. In contrast, an initial decrease in QoL score, followed by better scores, was achieved by those with stage II and III, maybe because of a better perception of QoL after diagnosing CRC (Paika et al, 2010). In patients with CRC undergoing surgery and colostomy, such procedures could lead to physical, psychological and social consequences on QoL.

Studies showed an immediate decrease in QoL scores after surgical interventions, and a gradual recovery after 3 months. The presence of colostomy can lead to decreased QoL physical scores but also to emotional and social functioning scores as compared to those in patients with resection and anastomosis, but not all authors registered a significant difference (Tafreshi et al, 2010).

Various studies worldwide investigated the role of chemotherapy and its associated symptoms influencing QoL in patients diagnosed and treated for CRC (Gray et al, 2011). In Romania, the risk of developing cancer before the age of 75 is 23.1%, of which 28.0% in men and 19.1% in women. The risk of dying from a form of cancer is 14.0%, of which 19.1% in men and 9.8% in women. Colorectal cancer is the second most common cancer that affects the Romanian population (21,387,000 inhabitants), for both men and women.

The number of new CRC cases reported in 2012 was 10,256 (13% of all cancers), of which 5760 were men (13.3% of all cancers in men) and 4496 were women (12.6% of all cancers in women). In 2012 the number of deaths due to CRC was 5675 people, of which 3229 were men and 2446 were women. The 5-year prevalence was 24,170 cases of CRC, of which 13,654 were men and 10,516 were women (Ferlay et al, 2018). Of all the neoplastic sites, CRC is one of the most common familial cancers. People with Lynch syndrome are prone to various types of cancer, with a predilection for colonic and endometrial damage. Lynch syndrome accounts for 2–4% of all cases of CRC. Affected individuals may develop colonic adenomas with a higher frequency than the general population. The lifetime risk of developing a CRC is estimated at 50% (Jasperson et al, 2010).

Lynch syndrome is the result of a germline mutation in a class of genes involved in DNA mismatch repair (MMR), including hMSH2, hMLH1, hMSH6 and hPMS2 (Rustgi et

al, 2007). The QoL questionnaire of the European Organization for Cancer Research and Treatment (EORTC) is an integrated type of tool used to assess the QoL of cancer patients from a health point of view (Jasperson et al, 2010). The aim of the study was to evaluate the QoL of patients with colorectal cancer with genetic risk who underwent preoperative cancer treatment (chemoradiation) and then underwent surgery, using an official questionnaire translated into Romanian.

Material and methods

The study group consisted of 32 patients who freely consented to participate in the research. The cross-sectional study was conducted between November 2019 and March 2020 and interrupted temporarily due to the COVID-19 pandemic lockdown in Romania on March, 16th, 2020. The study was carried out at Regional Institute of Oncology, Iasi, Romania.

The genetic risk was analyzed based on the genetic tree and the Amsterdam criteria by oncogenetics specialists. The inclusion criteria in the study were: patients over 18 years of age, without cognitive disorders, with unaltered judgment and introspection capacity, having awareness of oncological disease, with oncological diagnosis and genetic risk.

All patients who were asked to answer the questionnaire, after basic information of the study, gave their informed consent and filled in the QoL assessment questionnaire.

The exclusion criteria were patients with oncological diagnosis but without a genetic risk, those in the stage of denial of the oncological disease, confused or disoriented, and those with problems in understanding the instructions to fill in the questionnaire.

The study was conducted in accordance with the Helsinki Declaration and with several published principles (Lizdenis et al, 2015; Agheorghiesei et al, 2015; Toader et al, 2018; Toader et al, 2012; Toader et al, 2010; Agheorghiesei (Corodeanu) et al, 2016; Poroch et al, 2018). The inclusion criteria were suspected diagnosis of Lynch syndrome (Hereditary Nonpolyposis Colorectal Cancer), preoperative chemoradiation, and colostomy surgery.

The exclusion criteria referred to the diagnosis of sporadic CRC, but they were not related to the age group, gender, or residence area of the patients. Genetic risk was assessed taking into account the Amsterdam II Criteria (1998) for the identification of hereditary risk at CRC, also called criteria 3-2-1, as follows:

1. at least three persons in the same family affected by histologically confirmed cancer belonging to the HNPCC-Lynch spectrum (colorectal, endometrial, small bowel, urinary tract, renal pelvis, ovarian) histologically proven, united 2 by 2 by degree 1 of kinship (one of the persons is a 1st degree relative of the other two);
2. at least two generations are involved;
3. at least one of these cancers was declared before the age of 50 (familial adenomatous polyposis must be excluded).

The family history and the establishment of the genetic tree were traced (a key element for the evaluation of the notion of hereditary risk).

The features are provided in the Figure 4.2.

The chemotherapy protocol consisted either of monotherapy with fluoropyrimidines (5- fluorouracil intravenously; capecitabine or tegafur-uracil, both administered orally) in combination with preoperative radiotherapy, or as combination therapy (5-fluorouracil, leucovorine, and oxaliplatin).

The cross-sectional study consisted of applying the EORTC questionnaire, version QLQ-CR29 specially designed for patients with CRC.

The version translated in Romanian language was used online with the consent of the EORTC Organization, used for academic purposes only.

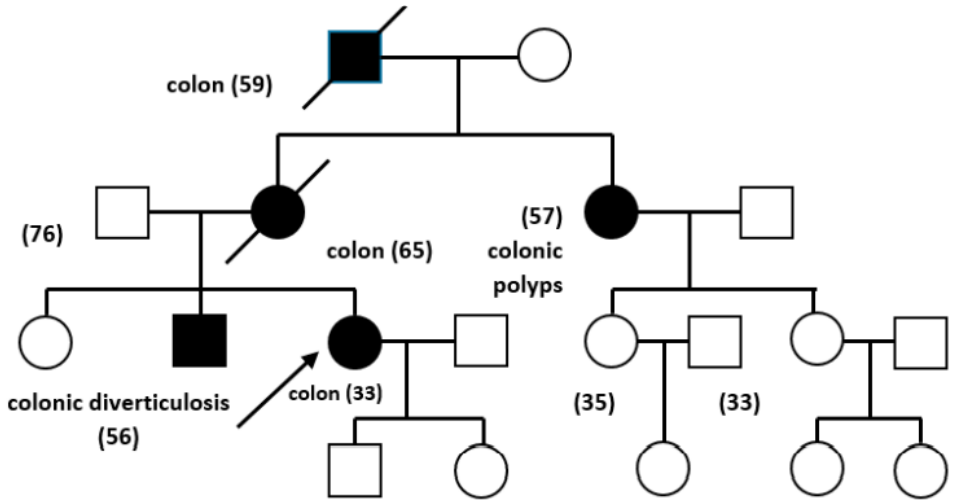

Fig. 4.2. The genetic tree of a patient included in the present study

The QLQ-CR29 questionnaire has not been validated yet on the Romanian population, this being the purpose of a subsequent research based on the results of the present study. As of now there is only the official translation of the questionnaire in Romanian accepted by EORTC.

Questionnaire validation for the study group. Cronbach's alpha coefficient value was calculated separately by gender, so Cronbach's alpha coefficient in males was 0.819 and females was 0.907. Both values are above the threshold required to validate the use of the questionnaire (minimum value = 0.700), thus the questionnaire was validated for the study group.

The principle of QLQ-CR29 scores consisted of applying to all cases the same questionnaire and the primary score was calculated for each case; the primary score was standardized into two types of scores used by QLQ-CR29; the standardized scores ranged from 29 to 114, where a higher score represents a better functioning.

The questionnaire subscales were the following: symptoms scale (scores ranged 15–30); functioning scale with the subsequent emotional scale (score ranged 5–20) and physical functioning including sex life scale (scores ranged 10–27).

Results

The demographic data and tumor localization are presented in Table 4.I.

Table 4.I. Demographic data and tumor localization

Variables	Statistics
Age	Range: 44–56 Mean: 49.18 Most frequent group: 45–50 (53.12%)
Gender	Male: 37.50% Female: 62.50% M: F ratio = 0.6
Residence area	Urban: 71.87% Rural: 28.13% U: R ratio = 2.55
Educational level	Compulsory: 15.62% Upper secondary: 37.50% Higher: 46.88%
Tumor localization	Sigmoid: 40.62% Rectosigmoid: 28.13% Rectal: 31.25%

Symptoms scale answers were added in Table 4.II.

Table 4.II. Symptoms scale

Items	Not at All		A Little		Quite a Bit		Very Much	
I31. Urinary frequency/day	N = 2 6.30%	M = 50% F = 50%	N = 17 53.1	M = 70.6% F = 29,4%	N = 13 40.60%	M = 53.8% F = 46.2%	N = 0 0.00%	M = 0.0% F = 0.0%
I32. Urinary frequency/night	N = 6 18.80%	M = 83.3% F = 16.7%	N = 16 50	M = 81.3% F = 18.7%	N = 9 28.10%	M = 24.2% F = 77.8%	N = 1 3.10%	M = 0.0% F = 100.0%
I33. Urinary incontinence	N = 23 71.90%	M = 65.2% F = 34.8%	N = 9 28.10%	M = 55.6% F = 4.4%	N = 0 0.00%	M = 0.0% F = 0.0%	N = 0 0.00%	M = 0.0% F = 0.0%
I34. Dysuria	N = 20 59.40%	M = 52.6% F = 7.4%	N = 9 28.10%	M = 77.8% F = 22.2%	N = 4 12.50%	M = 75.0% F = 25.0%	N = 0 0.00%	M = 0.0% F = 0.0%
I35. Abdominal pain	N = 13 40.60%	M = 53.8% F = 46.2%	N = 6 37.50%	M = 73.3% F = 26.7%	N = 12 18.80%	M = 83.3% F = 16.7%	N = 1 3.10%	M = 0.0% F = 100.0%
I36. Buttock pain	N = 12 37.50%	M = 41.7% F = 58.3%	N = 15 46.90%	M = 73.3% F = 26.7%	N = 5 15.60%	M = 80.0% F = 20.0%	N = 0 0.00%	M = 0.0% F = 0.0%
I37. Bloated feeling	N = 6 18.10%	M = 33.3% F = 66.7%	N = 19 78.15	M = 63.2% F = 36.8%	N = 7 21.90%	M = 85.7% F = 14.3%	N = 0 0.00%	M = 0.0% F = 0.0%
I38. Blood in stools	N = 16 50.00%	M = 50% F = 50%	N = 14 43.70%	M = 78.6% F = 21.4%	N = 2 6.30%	M = 50% F = 50%	N = 0 0.00%	M = 0.0% F = 0.0%
I39. Mucus in stools	N = 16 50.00%	M = 56.3% F = 43.8%	N = 16 50.00%	M = 58.8% F = 41.2%	N = 0 0.00%	M = 0.0% F = 0.0%	N = 0 0.00%	M = 0.0% F = 0.0%
I40. Dry mouth	N = 4 12.50%	M = 0.0% F = 100.0%	N = 20 62.50%	M = 60.0% F = 40.0%	N = 6 18.80%	M = 50% F = 50%	N = 2 6.30%	M = 50% F = 50%
I41. Hair loss	N = 8 25.00%	M = 37.5% F = 62.5%	N = 13 40.60%	M = 76.9% F = 23.1%	N = 7 21.90%	M = 71% F = 28.6%	N = 4 12.50%	M = 50% F = 50%
I42. Trouble with taste	N = 10 31.30%	M = 50% F = 50%	N = 12 37.50%	M = 75.0% F = 25.0%	N = 9 28.10%	M = 55.6% F = 44.4%	N = 1 3.10%	M = 100.0% F = 0.0%

By applying the Kruskal–Wallis significance tests, the correlation matrix of the items for symptoms scale highlighted the following aspects of significance for the purpose of our study: urination at night correlated significantly with abdominal pain ($p = 0.015$) and loss of taste ($p = 0.011$); urination pain was significantly correlated with abdominal pain ($p = 0.002$), blood stool ($p = 0.007$), and loss of taste ($p = 0.017$); the bloating sensation was significantly correlated with hair loss ($p = 0.021$) and taste loss ($p = 0.05$); the presence of blood in stools was significantly correlated with hair loss ($p = 0.047$) and taste loss ($p = 0.03$); hair loss was significantly correlated with taste loss ($p = 0.037$).

The symptoms scores values ranged from 15 to 30, with a mean of 22.47; the mean level did not differ significantly between genders (22.0 vs. 22.75; $p = 0.636$), showing a moderate impairment of the QoL (Table 4.III and Figure 4.3).

Table 4.III. Descriptive statistics of symptoms score by gender

Gender	N	Mean	Std. Deviation	Std. Error	95%Confidence Interval		Min	Max	F Test (ANOVA)
					−95%CI	+95%CI			*p*
Male	12	22.00	4.02	1.16	19.44	24.56	17	28	0.636
Female	20	22.75	4.45	0.99	20.67	24.83	15	30	
Total	32	22.47	4.24	0.75	20.94	24.00	15	30	

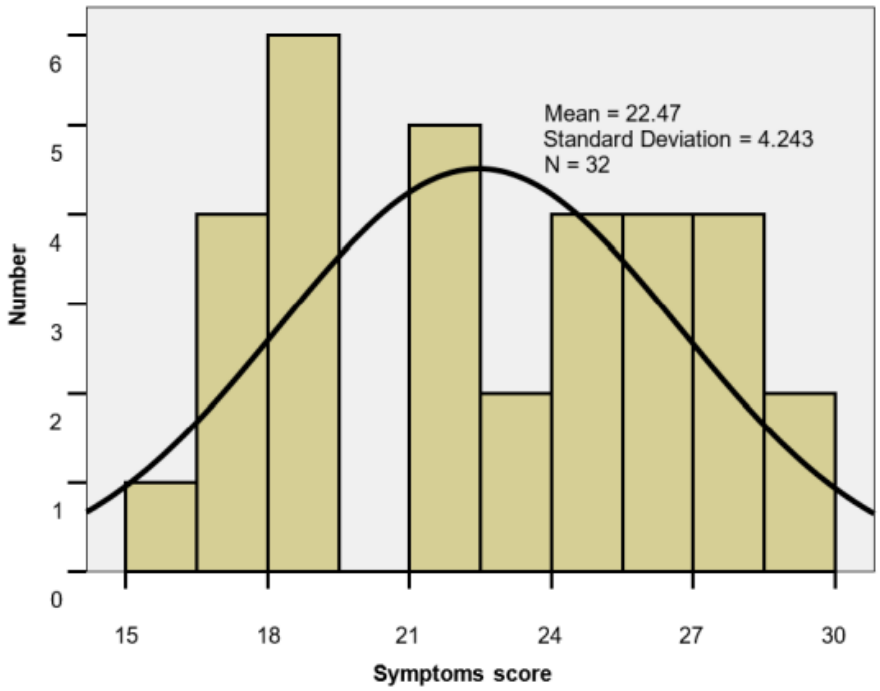

Fig. 4.3. Symptoms score histogram

Emotional functioning scale answers

By applying Kruskal–Wallis significance tests, the correlation matrix of items for emotional functioning scale revealed strong and statistically significant correlations between the patient's worries for future health, weight, attractiveness, and masculinity/femininity, generally related to one's body ($p < 0.05$).

Emotional Functioning Score

The scores values for emotional functioning scale ranged between 5 and 20. The scores values were homogeneous, with the mean level lower in men than in women (10.33 vs. 13.25; $p = 0.049$), highlighting a decrease in QoL of patients suffering from CRC.

Physical Functioning Scale

By applying the Kruskal–Wallis significance tests, the correlation matrix of items for physical functioning scale in the QLQ-CR29 questionnaire highlighted the following significant aspects for our study: stool leakage was significantly correlated with a sore skin ($p = 0.001$), an accelerated intestinal transit during day ($p = 0.024$) or night ($p = 0.047$) and with the embarrassment to wear a stoma bag ($p = 0.018$), and with a decreased interest for sex ($p = 0.006$).

Physical Functioning Score

The scores values for the physical functioning scale ranged 10–27; the group mean 17.84 ± 4.66 was close to the median value of 18. The scores mean level was significantly lower in men (15.67 vs. 19.15; $p = 0.039$), indicating an important impairment of physical functioning.

Discussions

The modern approach of treatment options for patients with CRC leads to the necessity of taking into consideration their QoL after surgical and/or oncological protocol application.

QoL should be included in the evaluation of such patients, along with the survival assessment, tumor recurrence, side effects and toxicity of chemoradiation, the physical and psychological effects of wearing a stoma, as well as the cost-effectiveness of procedures.

We consider that the main statements of our study are the following: the average level of scores for symptoms did not differ significantly between the sexes, highlighting a

moderate impairment of QoL. Men did not feel less masculine, while women felt less feminine as a result of illness and treatment; the correlation matrix of items for emotional function showed strong correlations, statistically significant between the patient's concerns for future health, weight, feelings of dissatisfaction with attractiveness, femininity/masculinity and, generally, with their own body; the symptoms characteristic of discomfort caused by the colostomy bag or unintentional manifestations generated by bowel movement showed correlations that led the patient to reanalyse his/her physical and emotional effects, and greater impairment of physical and emotional functions was noted in men. Scores are transformed linearly, giving a score between 0 and 100.

The results of our study were compared to the results of other studies that showed that the higher the scores, the better they function on the functioning scales and a higher level of symptoms on the symptoms scale (van der Hout et al, 2019).

The results of the present study will be used in the calibration of the Romanian population and the construction of their own standards for patients with CRC with a genetic risk.

The physical and emotional changes in patients with stoma varied by gender. In female patients, a lower QoL score was reported for emotional and physical functioning, but in males, we described, just like other studies (Reese et al, 2013), a decrease in the score. These findings can lead to a gradual reduction in a person's confidence and can change his/her social functioning as well (Marventano, 2013).

The impact of colostomy, together with chemoradiation and other therapeutic procedures in patients diagnosed with CRC, could be ameliorated by providing specialized assistance, such as colostomy care programs and psychological counseling (Vonk-Klaassen et al, 2015).

In a study on patients with CRC and their partners, regarding the influence of oncological treatments on psychological functioning, the authors showed significant differences in anxiety, depression and traumatic stress symptoms among patients who underwent surgery, and chemoradiation, as compared to patients treated only surgically. The first group experienced higher anxiety scores, more depressive symptoms and more posttraumatic stress symptoms (Pereira et al, 2012).

The limitations of the QoL studies in patients with CRC could be the lack of accumulation of information by systematic reviews and the lack of a gold standard for QoL measurement.

The strengths of the present study refer to a group of patients with Lynch syndrome who are young patients, socially active, for whom to maintain a high level of QoL is extremely important even after the oncological therapeutic interventions. The weaknesses of our study refer to the small group, few demographic variables and the non-inclusion in the statistical evaluation of the histopathological and surgical data.

The same team of researchers from our university intends to continue the study on larger groups of patients, in order to better conclude the results and extrapolate the conclusions in the hospital management, population awareness and patients' information, as well as for future projects of other research teams.

Conclusions

The effects of chemoradiation associated with colostomy in colorectal cancer patients revealed correlations that made the patient to re-analyse the physical and emotional functioning.

In our study, impairments of physical and emotional functioning scores were observed. The global score highlighted a moderate decrease in quality of life, both sexes being equally affected.

SECTION II - Career perspectives

The second section of this habilitation thesis outlines short, medium as well as long-term research directions and development projects, concentrating on the comprehensive and challenging interrelation between professional, academic and scientific research.

II.1. Plans for the development of scientific and professional activity

On short, medium and long-term, my research activities will be guided toward:

- Continuing and expanding the collaboration with the preclinical disciplines such as Immunology, Biochemistry, Pharmacology and other dental disciplines (Prevention, Endodontics, Periodontology), addressing new proposals that could provide real funding through joint research projects;
- Expanding collaboration with "Petru Poni" Institute of Macromolecular Chemistry;
- Attracting funds to improve the means of research at the Internal Medicine discipline;
- Organizing and participating in national and international scientific events;
- Developing applications for international research programs and grants;
- Organization of scientific events, with the involvement of the PhD students for improving their scientific knowledge;
- Dissemination of results in ISI / indexed international databases journals.

In long term, an important goal will be to participate in conferences, symposia, congresses, at European level in order to develop interaction with opinion leaders in the field, continuous updating of specialized information in order to improve medical, didactic and research. In the future, I will also seek to intensify the publishing activity in journals with a high impact factor, so that in this way I can acquire, together with colleagues, the entire department and, of course, our university, as much visibility as possible nationally and internationally. Workshop events, training, exchanges of experience with prestigious partners will provide the basis for continuous individual training, these activities will give me the opportunity to access information of immediate interest and with the potential for rapid further development, which will be able to materialize in new projects and research topics.

II.2. Future plans for the academic activity progress

The future development of the university career has as main objective the increase of the quality of the didactic process and the continuation of the professional development by meeting the standards of an educational process of excellence, the accumulation of new knowledge, skills and abilities related to teaching, clinical and research activities, all through continuous training. A systematization of these objectives includes:

1. Development of teaching activity;
2. Development of scientific research activity;
3. Development on a medical line;
4. Institutional development.

In the development of the educational activity, I consider as main objectives the increase of the efficiency and quality of the educational process. In this regard, my proposals believe that I can contribute to the fulfilment of these goals:

Thorough training of doctoral students, from a scientific and practical point of view

Educating students and doctors resident in the formative spirit, by using interactive teaching methods, characterized by case study, simulation, role play, all these methods

having the role of capturing them and maintaining interest in quotas that allow an efficient accumulation of medical information.

Accepting and encouraging the teacher-student / resident / doctoral student dialogue, alternative points of view, constructive criticism and encouraging the elaboration of personal solutions. The teacher must have the availability to respond to the curiosities and expectations of knowledge and education of students, while aiming to cover the curricular objectives.

Receptivity to the individual needs of students, residents, as well as doctoral students – professional, scientific, personal, with the role of developing long-term professional relationships between coordinator and student. Improving communication with students, either directly (report), or electronically in order to obtain a feedback of the teaching activity and to guide their individual study;

Continuous updating of the content of courses and practical works, in accordance with the evolution of knowledge in Internal Medicine and Geriatrics, based on documentation and results obtained in research activity. The teaching material must consist mainly of schemes, graphics, figures, pictures, diagrams, films, info grams that attract the interest of students and keep their attention on the topics presented;

Mostly use of modern means of presentation (computer / projector / course media). A priority will be the modernization of teaching methods used through the intensive use of state-of-the-art technological means;

Carrying out in the discipline, in collaboration with all colleagues, a guide to practical works by Internal Medicine and Geriatrics, in which information is focused on clinical cases and combines multidisciplinary information, thus encouraging both the diagnostic and therapeutic reasoning of students.

Participation, as a lecturer, in various scientific events, postgraduate courses;

Permanent didactic education of resident doctors and doctoral students in postgraduate courses, case presentations through modern, interactive methods that stimulate them in the learning process. In the process of continuous improvement, modern in format, I do not think that the permanent confrontation of theoretical notions with clinical reality must be minimized, for the training of good professionals, being still valuable the closeness to the patient, for the full knowledge of the complexity of the nature of the human body. Last but not least, the knowledge and differentiated counselling of students must be considered through meetings on learning, career guidance, social assistance, in tutoring issues.

Determination of the main future research directions in accordance with the latest topics of interest in the interdisciplinary fields as Internal Medicine make possible and only in research directions whose results can have applicability in medical practice.

Future scientific work will focus primarily on areas and research areas that are already active, including tasks that have been the focus of my attention for the last years. The pandemic taught us to live with the virus but how the SARS-CoV-2 infection has influenced the clinical and therapeutic approach, and which are the new long Covid-19 challenges, are questions that need future research.

The complex metabolic syndrome, metabolic fatty liver and intestinal microbiota remains an permanent challenge for the scientific community. There is intricate relationship between NAFLD, type 2 diabetes, obesity, and the continuous growing of obesity and diabetic people that rise concerns and need deep and continuous studies in this area.

A new approach into immunogenicity and novel TNF-α inhibitors or, old drugs - new challenges are also topics of interest for me that will need further studies.

A new research direction is focused on Age-Related Diseases and Clinical and Public Health Implications. The percentage of national populations over age 65 has been increasing in the last 10 years and will continue to rise for another 20 years due to improved life

expectancies. There are a wide variety of age-related changes in the immune system, some mediated by chronic inflammation and a chronic pro-inflammatory state. There is a decline in B cell function, a decline in T cell generation, altered T cell activation, and dysfunction of innate immunity (including impaired neutrophil function and chemotaxis and a dysregulated proinflammatory monocyte response). There are few studies in this area and that is way I want to deepen this direction of research.

Thanks to recent advances in computer science and informatics, artificial intelligence (AI) is quickly becoming an integral part of modern healthcare. AI algorithms and other applications powered by AI are being used to support medical professionals in clinical settings and in ongoing research.

Currently, the most common roles for AI in medical settings are clinical decision support and imaging analysis. Clinical decision support tools help providers make decisions about treatments, medications, mental health and other patient needs by providing them with quick access to information or research that's relevant to their patient. The challenges that the COVID-19 pandemic created for many health systems also led many healthcare organizations around the world to start field-testing new AI-supported technologies, such as algorithms designed to help monitor patients and AI-powered tools to screen COVID-19 patients.

The research and results of these tests are still being gathered, and the overall standards for the use AI in medicine are still being defined. Yet opportunities for AI to benefit clinicians, researchers and the patients they serve are steadily increasing. At this point, there is little doubt that AI will become a core part of the digital health systems that shape and support modern medicine.

In conclusion, as an overview of my career, I can say that hard work, perseverance, curiosity, communication skills, and teamwork are the keys to becoming a successful educator, researcher, and clinician.

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