UNIVERSITATEA LUCIAN BLAGA DIN SIBIU

ABSTRACT OF HABILITATION THESIS

RESULTS OF CLINICAL AND BIOMEDICAL RESEARCH: FROM THERAPEUTIC APPROACHES IN CATASTROPHIC ANTIPHOSPHOLIPID SYNDROME TO INNOVATIVE NANOCOMPOUNDS AND LABORATORY TECHNIQUES

LIANA MARIA CHICEA, Md., PhD.

SIBIU 2024

Table of Contents

1.SCIENTIFIC CONTRIBUTION AND RESULTS

1.1.Contribution and Results in Clinical Studies

1.1.1.Therapeutic Plasma Exchange in Catastrophic Antiphospholipid Syndrome

Catastrophic antiphospholipid syndrome (CAPS) is a critical, life-threatening subset of antiphospholipid syndrome, characterized by rapid onset of multiple organ thromboses and high mortality rates. This subchapter is grounded in the published paper "Therapeutic Plasma Exchange in Catastrophic Antiphospholipid Syndrome (CAPS): A Rare Case Report and Literature Review." Our study emphasizes the essential role of Therapeutic Plasma Exchange (TPE) as a first-line treatment intervention in CAPS. TPE involves the extracorporeal removal of plasma containing pathogenic substances such as autoantibodies and its replacement with a suitable fluid. The case report focuses on an elderly patient with CAPS secondary to systemic lupus erythematosus (SLE), highlighting the successful use of TPE alongside glucocorticoids, cyclophosphamide, and anticoagulants. The patient's improvement post-TPE included the stabilization of hemodynamic parameters, normalization of blood counts, and resolution of coagulation abnormalities. This comprehensive approach demonstrates the significant impact of early and aggressive therapeutic interventions in improving survival and quality of life in CAPS patients.

1.1.2.Off-Label Use of Tocilizumab and Adjuvant Iron Chelator Therapy in COVID-19

The outbreak of COVID-19 presented numerous challenges, particularly in managing severe cases characterized by hyperinflammatory responses or "cytokine storms." This section, based on the paper "Off-label Tocilizumab and Adjuvant Iron Chelator Effectiveness in a Group of Severe COVID-19 Pneumonia Patients: A Single Center Experience," explores the off-label use of Tocilizumab (TCZ), an IL-6 receptor antagonist, combined with iron chelation therapy using deferasirox in critically ill patients. This retrospective observational study, conducted at the Academic Emergency Hospital Sibiu, evaluates the clinical characteristics, treatment regimens, and outcomes of 25 patients with severe COVID-19 pneumonia. Administration of TCZ and deferasirox showed promising results, with improvements in oxygen saturation, reductions in inflammatory markers such as C-reactive protein (CRP) and IL-6, and better overall clinical stabilization. The study reveals that timely intervention with TCZ and iron chelation can significantly mitigate the adverse effects of severe inflammatory responses and enhance patient recovery in severe COVID-19 cases.

1.1.3.Impact of SARS-CoV-2 Infection on Maternal and Neonatal Outcome

In this research, the effects of SARS-CoV-2 on pregnancy and neonatal outcomes are investigated. This retrospective study involves 398 pregnant women, half of whom tested positive for SARS-CoV-2, and examines the virus's impact during different pandemic phases, including the dominance of the Delta and Omicron variants. The study identifies key sociodemographic factors such as education, employment, and residence, correlating them with maternal and neonatal health

outcomes. Despite initial concerns, the research finds no significant increase in adverse neonatal outcomes or preterm births among infected mothers when essential medical care and individualized treatment strategies are provided. This analysis provides reassurance regarding the safety of maternal SARS-CoV-2 infection and highlights the importance of individualized care and access to healthcare services for better maternal and neonatal outcomes.

1.1.4.Dose-dependent Spasmolytic, Bronchodilator, and Hypotensive Activities of Panicum miliaceum

Proso millet (Panicum miliaceum L.) has a long tradition of use in treating various ailments. This subchapter, based on the published paper "Dose-dependent Spasmolytic, Bronchodilator, and Hypotensive Activities of Panicum miliaceum L.," investigates its scientific basis as a therapeutic agent. The study employed in vitro assays using isolated rabbit jejunum, trachea, and aorta tissues to explore the extract's dose-dependent effects. Findings indicate that Panicum miliaceum exhibits significant spasmolytic, bronchodilator, and hypotensive activities, primarily through mechanisms involving potassium channel opening. This supports its traditional use in managing gastrointestinal spasms, respiratory conditions, and hypertension. The research identifies multiple bioactive compounds responsible for these effects, suggesting that Panicum miliaceum can be a potent natural remedy for various physiological disorders.

1.1.5.Metabolomics Insights to Vasorelaxant and Cardioprotective Effect of Citrullus lanatus Seeds in Myocardial Infarction

In this subchapter, based on the study "Metabolomics-based Mechanistic Insights to Vasorelaxant and Cardioprotective Effect of Ethanolic Extract of Citrullus lanatus (Thunb.) Matsum. & Nakai Seeds in Isoproterenol Induced Myocardial Infarction," we explore the cardioprotective potential of Citrullus lanatus, commonly known as watermelon. The research employs metabolomic profiling to unravel the bioactive compounds and pathways through which the seeds exert vasorelaxant and cardioprotective effects. The study demonstrates that the ethanolic extract of Citrullus lanatus seeds significantly mitigates isoproterenol-induced myocardial infarction by promoting vasorelaxation, improving energy metabolism, and reducing oxidative stress. Key bioactive compounds identified, such as quercetin, kaempferol, and rutin, are linked to the observed therapeutic effects. The study's in vitro and in vivo experiments confirm the efficacy of these compounds in enhancing myocardial function and reducing infarction size, positioning Citrullus lanatus seeds as a valuable natural intervention in cardiovascular health.

1.1.6.The therapeutic effects of Cucumis sativus L. seeds extract of on hypertension and myocardial infarction

This chapter presents a detailed investigation of the therapeutic effects of an extract derived from the seeds of Cucumis sativus L. on hypertension and myocardial infarction. Utilizing a metabolomics approach, the study aimed to elucidate the protective mechanisms of this hydroethanolic extract in experimental models of hypertension and isoproterenol-induced myocardial infarction.

The process of preparing the hydroethanolic extract involved meticulously collecting and processing seeds of Cucumis sativus, followed by extraction using a specialized Soxhlet apparatus with a specific hydroethanolic solution consisting of 70% ethanol and 30% water. Animal models

including albino rabbits, Sprague-Dawley rats, and mice were utilized in the study, with all experimental procedures being ethically approved by the Bahauddin Zakariya University Ethical Committee and adhering to ARRIVE guidelines and national regulations for animal research.

Experimental protocols encompassing isolated tissue experiments were employed to evaluate the vasorelaxant and cardioprotective properties of the hydroethanolic extract. These experiments entailed utilizing isolated aorta and paired atria to assess the physiological responses to the extract based on established methodologies from previous studies.

In vivo experimentation involving varying doses of the extract administered to different groups of rats over a specified duration enabled the assessment of its maximum tolerable dose and effects on parameters such as blood pressure and hemodynamics under normotensive and L-NAME-induced hypertensive conditions. The results of these experiments were crucial in unveiling the potential antihypertensive properties of the extract.

Furthermore, the identification of bioactive compounds within the hydroethanolic extract through LC-ESI-MS/MS analysis shed light on its composition, which included noteworthy compounds such as chlorogenic acid, gallic acid, catechins, and rutin. These compounds are known for their antioxidant, anti-inflammatory, and vasoprotective properties, suggesting their role in the observed therapeutic effects of the extract.

Significant findings from the study revealed the extract's abilities to induce vasorelaxation in both endothelium-intact and denuded aortic preparations, along with its antihypertensive effects demonstrated in experimental models. The cardioprotective effects of the extract were particularly highlighted in isoproterenol-induced myocardial infarction models, where it showcased improvements in biometrical indices and reductions in inflammatory markers, emphasizing its potential in protecting cardiac tissue from ischemic injury.

Moreover, a comprehensive metabolomics analysis provided valuable insights into the modulation of key metabolic pathways associated with energy production, oxidative stress, amino acid metabolism, and lipid metabolism induced by the extract. These metabolic alterations indicated enhancements in cardiac energy metabolism and reductions in myocardial stress, reinforcing the extract's cardioprotective mechanisms.

1.1.7.Assessment of Hypothalamic-Pituitary-Adrenal Axis Function Using High-Dose Cosyntropin Testing in Patients with Low-Dose Stimulation Abnormalities

This chapter presents an exploration of adrenal function assessment using low-dose (1 μg) and high-dose (250 μg) ACTH stimulation tests in diagnosing secondary adrenal insufficiency (SAI). Their central argument challenges the standard practice of relying on high-dose testing by proposing that the low-dose test might represent a more accurate and potentially less invasive diagnostic tool, particularly for detecting partial AI cases.

In this study, a cohort of 26 patients with suspected SAI underwent both the low-dose and high-dose tests. Blood samples were collected before and after ACTH administration to measure cortisol levels, serving as a primary indicator of adrenal function. Notably, all participants failed the low-dose test, displaying inadequate cortisol responses. However, a significant portion of the cohort (23 out of 26) exhibited normalized cortisol responses following the high-dose test, suggesting a distinct efficacy in diagnosing SAI when compared to the low-dose protocol.

Further analysis revealed uniform basal cortisol levels across both testing methods, failing to provide diagnostic differentiation for AI in most cases. However, a crucial disparity emerged in cortisol levels at the 30-minute mark, showcasing significantly lower readings in the low-dose test compared to the 30 and 60-minute readings in the high-dose test. Adjusting the threshold for a normal response in the high-dose test to 20 μg/dL yielded only two additional failures, underlining

the sensitivity and specificity of this approach. It's particularly noteworthy that all patients failing the low-dose test demonstrated normalized cortisol responses with the high-dose variant, emphasizing the latter's superior diagnostic capabilities.

In conclusion, we advocate for the recognition of the low-dose test as a dependable and sensitive diagnostic tool for identifying SAI. We highlight the simplicity and enhanced safety profile of the low-dose protocol, showcasing potential benefits such as reduced risk of overstimulation in partially atrophied adrenal glands. While acknowledging the study's limitations, including its retrospective design and limited sample size, we emphasize the urgent need for further research to validate and optimize the utilization of the low-dose test in effectively diagnosing SAI in clinical practice.

1.2.Studies on Physiological and antibacterial effects of plant extracts and nanocomposites

1.2.1.On Cardio Protective and Hypotensive Activities of Anogeissus acuminata

This chapter presents a study on the cardio protective and hypotensive activities of Anogeissus acuminata. Traditional medicine in Southeast Asia has used this plant to manage cardiovascular disorders, indicating possible cardioprotective and hypotensive effects. The study aimed to validate these claims and explore the mechanisms behind Anogeissus acuminata's effects on cardiovascular diseases.

Analytical techniques like High-Performance Liquid Chromatography confirmed the presence of bioactive compounds in Anogeissus acuminata that have therapeutic properties related to cardiovascular health.

In vitro and ex vivo experiments demonstrated the cardiodepressant and vasorelaxant effects of Anogeissus acuminata, suggesting its potential in managing conditions like hypertension. In vivo studies showed that Anogeissus acuminata significantly lowered blood pressure and improved markers of cardiac hypertrophy, indicating its promising hypotensive and cardioprotective properties.

Histopathological analysis further supported the benefits of Anogeissus acuminata in mitigating cardiac remodeling, reducing inflammation, and protecting cardiac tissue integrity. Overall, the study concluded that Anogeissus acuminata exhibits potent cardioprotective and hypotensive properties, potentially offering an effective therapeutic approach for cardiovascular diseases like hypertension and myocardial infarction.

1.2.2.Antioxidant and Anticholinesterase Activity of Pimpinella anisum L.

Alzheimer's disease (AD) is a prevalent neurodegenerative condition characterized by cognitive decline and memory impairment, particularly affecting the elderly population. The degeneration of cholinergic neurons and oxidative stress are crucial factors in AD pathogenesis, contributing to neural damage and cognitive decline. The overactivity of the enzyme acetylcholinesterase (AChE) leads to the breakdown of acetylcholine (ACh) in the brain, exacerbating cholinergic deficits. Current treatments with AChE inhibitors have limitations, prompting the search for alternative therapies. Natural products, rich in antioxidants and anti-inflammatory agents, represent promising sources for AD management.

The study focused on Pimpinella anisum (anise), a medicinal plant rich in bioactive compounds known for their potential anticholinesterase properties. Aqueous extracts of P. anisum seeds were fractionated using column chromatography to isolate bioactive fractions. One particular fraction, F-8, demonstrated significant anticholinesterase activity, subsequently identified as the Pimpinella anisum active fraction (P.aAF). Chemical analysis using Gas Chromatography-Mass Spectrometry (GC-MS) identified several bioactive compounds in P.aAF, particularly oxadiazoles known for their diverse pharmacological activities, including antioxidant and anti-Alzheimer's effects.

In vivo studies in mice models revealed that P.aAF improved memory and cognitive functions, validated through behavioral tests like the elevated plus maze and biochemical analyses measuring antioxidative and cholinergic biomarkers. P.aAF-treated mice exhibited elevated levels of antioxidants, reduced acetylcholinesterase activity, and enhanced memory retention, indicating promising neuroprotective effects.

The study reveals that Pimpinella anisum L. possesses potent antioxidant and anticholinesterase activities, mainly attributed to its oxadiazole derivatives. The bioactive fraction, P.aAF, demonstrated significant cognitive enhancement and antioxidative effects in mice models. These findings suggest the therapeutic potential of P.aAF in treating AD and other neurodegenerative disorders. Further clinical trials are necessary to validate its efficacy and safety in human subjects, emphasizing the need for continued research to explore its neuroprotective mechanisms fully.

1.2.3.Advances in Noninvasive Diagnosis of Endometriosis

Endometriosis is a prevalent gynecological disorder affecting numerous women during their reproductive years. The condition, characterized by the presence of endometrial-like tissue outside the uterus, leads to complications such as chronic pelvic pain, dysmenorrhea, and infertility. Diagnosing endometriosis is challenging due to its nonspecific symptoms and the reliance on invasive laparoscopic surgery for definitive confirmation. The necessity for noninvasive biomarkers that enable early detection and continuous monitoring is crucial. This review aims to consolidate existing literature on potential biomarkers for endometriosis to facilitate improved diagnosis and management.

The pathophysiology of endometriosis involves various interconnected mechanisms, including retrograde menstruation, immune dysfunction, angiogenesis, oxidative stress, and hormonal imbalances. Retrograde menstruation theory explains the implantation of endometrial cells outside the uterus during menstruation but doesn't clarify why only a fraction of women develop endometriosis. Immune system dysregulation, characterized by abnormal immune responses and inflammatory mediators, contributes to the survival and proliferation of ectopic endometrial tissue. Oxidative stress and angiogenesis play significant roles in the growth and dissemination of endometriotic lesions.

A systematic literature review was conducted to identify and evaluate studies on noninvasive biomarkers for endometriosis. Various databases were searched from 2000 to 2019 using specific keywords related to biomarkers, angiogenesis, cytokines, and endometriosis. Inclusion criteria focused on peer-reviewed English articles examining biomarkers obtained from serum, plasma, or urine of human subjects in a noninvasive manner. A total of 55 studies meeting stringent criteria were reviewed in-depth.

Elevated levels of inflammatory markers such as C-reactive protein (CRP), interleukins (IL-6, IL-8, IL-1β), tumor necrosis factor-alpha (TNF-α), and macrophage migration inhibitory factor (MIF) characterize the inflammatory milieu in endometriosis. Studies highlight the diagnostic potential of IL-6, IL-8, TNF-α, and CRP as biomarkers for endometriosis, underscoring their roles in disease pathogenesis and progression.

Angiogenesis, essential for the growth of endometriotic lesions, involves vascular endothelial growth factor (VEGF) and its receptor (VEGFR). Oxidative stress markers like malondialdehyde (MDA) and superoxide dismutase (SOD) illustrate the imbalance in oxidative status in endometriosis, offering insights into disease progression and potential diagnostic utility.

Proteomic and genomic analyses have revealed distinctive protein profiles and genetic signatures associated with endometriosis. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have emerged as regulators with diagnostic potential due to their differential expression in affected individuals compared to healthy controls, highlighting their significance as biomarkers for endometriosis.

The comprehensive assessment of noninvasive biomarkers for endometriosis presented in this review paves the way for improved diagnostic strategies and personalized management approaches in the future.

1.2.4.Ag Nanoparticles for Biomedical Applications

The chapter delves into the versatile nature and promising applications of silver nanoparticles (Ag NPs) within diverse fields as outlined in the thesis. It discusses the unique properties of Ag NPs, including their optical, electrical, and antimicrobial characteristics, which have fueled significant interest within the scientific community. The research effort focuses on developing efficient and controlled synthesis methods for Ag NPs, leading to a comprehensive exploration of various approaches in the chapter.

Moreover, the chapter scrutinizes the crucial aspects of Ag NPs synthesis, highlighting the importance of size, shape, and surface modification in tailoring the nanoparticles for specific applications. It underscores the necessity of employing advanced characterization techniques such as Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and X-ray Diffraction (XRD) to analyze the structural and morphological features of Ag NPs, providing valuable insights for their utilization in various industries.

The chapter further elaborates on the diverse applications of Ag NPs, ranging from antibacterial agents to drug delivery vehicles, biosensors, catalysts, and electronic components. By exploring the synthesis methods, characterization techniques, and applications of Ag NPs comprehensively, this chapter paves the way for enhanced diagnostic strategies and personalized management approaches in multiple fields, showcasing the transformative potential of Ag NPs in scientific and industrial contexts.

1.2.5.Comparative Synthesis of Silver Nanoparticles

The chapter delves into the significance of nanomaterials, particularly silver nanoparticles (Ag NPs), and their applications in fields such as materials science, electronics, medicine, and environmental science due to their unique properties at the nanoscale. Silver nanoparticles are highlighted for their excellent electrical and thermal conductivity, along with remarkable antibacterial properties, making them attractive for advanced medical uses.

Various synthesis methods for Ag NPs are explored, including green chemistry approaches using plant extracts and chemical reduction methods. Green chemistry methods utilizing plant extracts have shown promising results, although challenges in reproducibility exist due to the diversity and growing conditions of plants. On the other hand, chemical synthesis methods offer advantages in terms of simplicity, cost-effectiveness, and high yield within a short timeframe. The chapter demonstrates the advancements in Ag NP synthesis and the importance of optimizing synthesis conditions for enhanced antibacterial effects.

Comparative studies on different synthesis methods shed light on the impact of synthesis conditions on the antibacterial activity of Ag NPs. Researchers have investigated the efficiency of

Ag NPs produced through various chemical synthesis methods, highlighting differences in particle size, shape, and antibacterial effects based on the synthesis route employed. The section emphasizes the need for precise control over synthesis parameters to tailor the properties of Ag NPs for specific applications effectively.

Characterization techniques such as Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS) play a crucial role in understanding the size, shape, and distribution of nanoparticles. These techniques provide valuable insights into the structural characteristics of Ag NPs synthesized through different methods, allowing researchers to optimize their performance based on specific requirements.

Overall, the chapter underscores the importance of synthesizing and characterizing nanoparticles effectively to harness their unique properties for advanced applications, particularly in antibacterial treatments and other cutting-edge technologies. The combination of diverse synthesis methods, comparative studies, and characterization techniques contributes to a comprehensive understanding of Ag NP synthesis and paves the way for their tailored use in various fields.

1.2.6.Silver Nanoparticles-Chitosan Nanocomposites

The chapter explores the development of silver nanoparticles-chitosan (AgNPs-CH) nanocomposites for antibacterial applications. It begins by discussing the significance of nanotechnology and biomaterials in modern medical advancements, focusing on combating infections and tissue engineering. Silver nanoparticles, known for their strong antibacterial properties, are combined with chitosan, a biodegradable polymer, to enhance biocompatibility and efficacy.

Research emphasizes the interaction of chitosan with AgNPs, forming robust nanocomposites with heightened antibacterial efficiency. Studies have shown that smaller nanocomposites exhibit more significant antibacterial effects, making them promising for various applications. Recent research showcases the enhanced antibacterial efficiency of AgNPs-CH composites synthesized using different methods and chitosan concentrations.

The chapter details the materials used and the methods employed for the synthesis of AgNPs-CH nanocomposites. Characterization techniques such as Atomic Force Microscopy (AFM), Dynamic Light Scattering (DLS), UV-VIS spectroscopy, and FT-IR spectroscopy were utilized to assess the properties of the nanocomposites.

Results illustrate that the nanocomposites have average diameters ranging from 40 to 100 nm, making them suitable for biomedical applications. UV-VIS spectroscopy confirmed the successful synthesis of silver nanoparticles, while FT-IR analysis validated the integration of AgNPs within the chitosan matrix. Antibacterial tests conducted on E. coli and S. aureus showed effective inhibition by the nanocomposites, with higher efficacy in samples with lower chitosan concentrations.

In conclusion, the study presents simple methods for synthesizing AgNPs-CH nanocomposites, showcasing their potential for antibacterial applications. The comprehensive characterization and antibacterial efficacy results suggest that these nanocomposites are promising candidates for combating bacterial infections efficiently.

1.3.Studies on Alternative Medical Laboratory Techniques

1.3.1.Light Scattering Dynamics Analysis of Biological Fluids

The chapter discusses the utilization of far-field interference fluctuations, known as speckle imaging, for characterizing dynamic behaviors in scattering media like fluid flow and sedimentation. Objective speckle, observed in free space, is the primary focus of the study. The

parameters of speckle, such as size, contrast, intensity, and polarization, provide valuable insights into scattering media characteristics.

The methodology involves recording and analyzing time series of far-field interference fluctuations at a single location using the autocorrelation function. An experimental setup featuring a laser, glass cuvette, detector, data acquisition system, and a PC is used. The study carefully addresses issues like turbulence and temperature differences to ensure reliable measurements.

The velocity of scattering centers in suspension, affected by sedimentation and Brownian motion, is calculated to understand the dynamic behavior of microparticles in the medium.

Results from analyzing 31 urine samples show that the autocorrelation time (ACT) can differentiate between healthy and pathological urine samples based on the type and amount of suspended particles. Samples with individual cells exhibit varying ACTs, with healthy urine having an ACT around 5 seconds. Conversely, samples containing conglomerates like oxalate crystals or albumin show increased ACTs due to larger particle masses.

The technique presented in the study offers a rapid and cost-effective screening tool for distinguishing between healthy and pathological urine samples based on particle characteristics. However, further specific laboratory tests are needed for comprehensive diagnosis as the method cannot differentiate between specific types of cells or proteins in the sample.

In conclusion, the developed technique using speckle imaging and autocorrelation analysis shows promise in detecting suspended particles in urine samples, enabling a quick initial screening for further detailed analysis.

1.3.2.Using Afm for Biological Samples Imaging

The chapter discusses the application of Atomic Force Microscopy (AFM) in imaging and analyzing biological samples, focusing on human serum albumin (HSA) molecules and Red Blood Cells (RBCs). AFM is highlighted for its ability to provide high-resolution images and detailed topography compared to standard optical microscopy.

The study first delves into the importance of understanding cell interactions with their environment and the significance of surface characteristics in influencing cell behavior. Nanoparticles are discussed for their compatibility with living cells, with applications ranging from cell investigations to drug delivery systems. The chapter emphasizes the need for techniques like AFM in studying cells and proteins due to their small sizes.

The experimental section details the principles and methodology behind AFM usage, specifically the Agilent 5500 AFM in ACAFM mode for imaging cells and proteins. Sample preparation and imaging techniques for RBCs and HSA are explained, focusing on creating single-layer samples for accurate visualization.

Results showcase optical microscopy images of RBC samples from healthy individuals and patients with spherocytosis, highlighting differences in RBC shapes and structures. AFM scans further reveal the biconcave structure of normal RBCs and the spherical morphology of RBCs in spherocytosis cases at higher resolutions.

The AFM imaging of HSA molecules on mica substrate demonstrates variations in protein concentration and dimensions, with extracted vertical profiles indicating albumin molecule sizes around 50 nm. The results align reasonably well with existing literature but show variations due to methodological differences and sample states.

The discussion compares AFM results with dynamic light scattering (DLS) and neutron scattering findings for HSA molecule sizes, suggesting the ellipsoid model for HSA molecules. Variations in size and orientation are attributed to sample preparation methods. Findings indicate a broad size distribution for HSA particles on mica, supporting the ellipsoid model over the spherical model.

The study underscores the utility of AFM for nanoscale biological imaging and measurement, showcasing its effectiveness in identifying cellular abnormalities like spherocytosis.

2.PROFESSIONAL, SCIENTIFIC AND ACADEMIC EVOLUTION AND INTENTIONS

Motto:
"Everything is possible, the impossible just takes longer."

2.1.Professional Evolution and Intentions

Education & Training:
- Graduated from General School No. 15 Sibiu (1979).
- Graduated from Octavian Goga Sibiu High School (1983), head of promotion.
- Graduated from the Faculty of General Medicine, University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj Napoca (1989) with an average of 9.97.
- Medical intern at Sibiu County Hospital (1990-1991), with internships in internal medicine, surgical specialties, and family planning.
- Passed the secondary school exam in 1991.
- Adult general medicine doctor at DMT 13 – Sibiu (1992).
- Secondary Internal Medicine doctor at Sibiu County Hospital and Clinica Medicală II Cluj-Napoca (1993-1995).
- Medical specialist in Internal Medicine (1995), confirmed by O.M.S.
- Primary physician in Internal Medicine (2000), confirmed by O.M.S.
- Resident doctor, 2nd Rheumatology specialty (2003-2005), and helped establish the Rheumatology Department in Sibiu in 2008.
- Became a Rheumatology specialist (2005) and primary doctor (2012).
- Worked continuously in Internal Medicine and Rheumatology, currently focusing mainly on Rheumatology since 2023.

Positions and Responsibilities:
- Coordinating physician of the Rheumatology Department (2008-2017).
- Head of Department Physician, Clinical Medical Department I, Sibiu County Clinical Hospital (2017-present).

Professional Training:
- Various professional training internships and postgraduate courses both in Romania and abroad.
- Multiple certifications and competencies in fields like family planning, palliative care, psychopedagogy, medical services management, general and clinical ultrasound, and musculoskeletal ultrasound.

Contributions and Achievements:
- Expanded diagnostic and treatment methods, offering a holistic view on complex medical problems.
- Played a significant role in establishing and developing the Rheumatology Department in Sibiu.

- Continuously updated with medical developments through participation in regional, national, and international medical events.

2.2.Scientific Evolution and Intentions

Research Initiatives:

- Research focus on metabolic syndrome and cardiovascular damage in patients with psoriatic arthritis.
- Use of the Romanian Register of Rheumatic Diseases (RRBR) for ongoing research.
- Exploration of Janus-kinase inhibitors (JAKi) in treating rheumatic inflammatory diseases.
- Developed interdisciplinary projects in various specialties like Dermatology, Gastroenterology, Ophthalmology, Pneumology, Pharmacology, Parasitology, Endocrinology, and Gynecology.

Collaborations and Trials:

- Principal investigator in more than 10 international multicenter clinical trials.
- Part of the Scientific Committee of the Romanian Society of Rheumatology (SRR) since 2015.
- Organized and supported multiple national events in Sibiu, including the Summer School of young rheumatologists and the National Congress of Rheumatology.

Publications:

- Rich publication record with a Hirsch index of 8, a cumulative impact factor of 46.64 for papers as the first author, and 209 citations according to WOS.

2.3.Academic Evolution and Intentions

T**eaching Career:**

- Started teaching in 1990 at Sibiu Health High School.
- Held positions of assistant professor and head of works between 1996 and 2014 at "Victor Papilian" Faculty of Medicine, "Lucian Blaga" University in Sibiu.
- University lecturer at the Medical Clinic Department of the Faculty of Medicine ULB Sibiu since 2014.
- Developed and taught courses in Internal Medicine, Medical Semiology, Medical Emergencies, Diabetes Care, Nutritional Diseases, and Rheumatology.
- Actively participated in organizing educational and examination events.

Academic Contributions:

- Participated in multiple local, regional, and national training programs and professional development activities.
- Developed course materials and proposed new courses for better student engagement and skill acquisition.
- Played a significant role in coordinating rheumatology residency programs and training medical professionals in rheumatology and related fields.

Future Goals:

- Promotion and enhancement of Rheumatology at the university.
- Increase in the teaching staff to address new specializations and higher demand for Rheumatology education.

- Continued development of interdisciplinary and scientific collaborations for better patient outcomes and academic research.
- Fostering new PhD projects to contribute to academic and medical fields.
- Creation and maintenance of a positive, cooperative academic environment for the growth of future specialists.

Conclusion

With extensive professional growth, teaching experience, and significant scientific contributions, this proposal aims to promote professional excellence, enhance patient care, and advance academic achievements in Rheumatology. The intention is to create a lasting legacy for patients, the hospital, faculty, and the medical community in Sibiu.

3.MY PUBLISHED PAPERS THAT SUPPORT MY THESIS

M1. Therapeutic Plasma Exchange in Catastrophic Antiphospholipid Syndrome (CAPS): A Rare Case Report and Literature Review, Bereanu, AS (Bereanu, Alina-simona), Pisaltu, T , Bereanu, R , Vintila, B, Codru, I., **Chicea, L,** Crisan, O., Cainap, C., Cainap, S., Sava, MIHAI, Journal IN VIVO Volume 37 Issue4 Pag e1914-1919, DOI10.21873/invivo.13286, 2023

M2. Off-label tocilizumab and adjuvant iron chelator effectiveness in a group of severe COVID-19 pneumonia patients A single center experience By: Birlutiu, V (Birlutiu, Victoria), Birlutiu, RM (Birlutiu, Rares Mircea), **Chicea, L** (Chicea, Liana) MEDICINE Volume100 Issue18, Article Number e2580 https://journals.lww.com/md-journal/fulltext/2021/05070/off_label_tocilizumab_and_adjuvant_iron_chelator.81.aspx, DOI10.1097/MD.0000000000025832, WOS:000658917700081, 2021

M3. Impact of SARS-CoV-2 Infection on Maternal and Neonatal Outcome in Correlation with Sociodemographic Aspects: A Retrospective Case-Control Study, by Radu Chicea, Andrei Dorin Neagu, Eugen Dan Chicea, Amina Simona Grindeanu, Dan Georgian Bratu, Adrian Gheorghe Boicean, Mihai Dan Roman, Sorin Radu Fleacă, **Liana Maria Chicea**, Dumitru Alin Teacoe, Ioana Andrada Radu and Maria Livia Ognean

M4. Dose-dependent Spasmolytic, Bronchodilator, and Hypotensive Activities of Panicum miliaceum L., Saqib, F (Saqib, Fatima); AL-Huqail, AA; Asma, M (Asma, Memona); **Chicea, L** (Chicea, Liana); Hogea, M (Hogea, Mircea)Irimie, M (Irimie, Marius); Gavris, C (Gavris, Claudia), DOSE-RESPONSE Volume 20 Issue 1, Article Number 15593258221079592,https://1510pirpa-y-https-journals-sagepub-com.z.e-nformation.ro/doi/10.1177/15593258221079592, DOI10.1177/15593258221079592, WOS:000776527300001

M5. Metabolomics based mechanistic insights to vasorelaxant and cardioprotective effect of ethanolic extract of Citrullus lanatus (Thunb.) Matsum. & Nakai. seeds in isoproterenol induced myocardial infraction, Saqib, F (Saqib, Fatima); Wahid, M (Wahid, Muqeet); AL-Huqail, AA (AL-Huqail, Arwa Abdulkreem); Ahmedah, HT (Ahmedah, Hanadi Talal); Bigiu, N (Bigiu, Nicusor); Irimie, M (Irimie, Marius); Moga, M (Moga, Marius); Marc, RA (Marc, Romina Alina); Pop, OL (Pop, Oana Lelia); **Chicea, LM** (Chicea, Liana Maria), Phytomedicine Volume 148, https://www.sciencedirect.com/science/article/pii/S0944711322001477, DOI10.1016/j.phymed.2022.154069, WOS:000794990600001, 2022

M6. Metabolomics based analysis delineates the therapeutic effects of hydroethanolic extract of Cucumis sativus L. seeds on hypertension and isoproterenol-induced myocardial infarction By:Wahid, M, Saqib, F, **Chicea, L**, Ahmedah, HT, Sajer, BH, Marc, RA, Pop, OL, Moga, M, Gavris, C, Biomedicine & pharmacotherapy Volume148 Article Number 112704 DOI10.1016/j.biopha.2022.112704 Published APR 2022

M7. Risk Factors Associated with Toxoplasma gondii in Patients with Cardiovascular Diseases from Western Romania. Dragomir A, Lupu MA, Maciuceanu CG, **Chicea LM**, Olariu TR, Microorganisms. 2024; 12(4):673. https://doi.org/10.3390/microorganisms12040673

M8. Seroprevalence and Associated Risk Factors of Toxoplasma gondii in Patients Diagnosed with Schizophrenia: A Case-Control Cross Sectional Study, Grada, S., Mihu, AG, Oatis, DA, Marc,

CC, **Chicea, LM**, Petrescu, C, Lupu, AM, Olariu, TR, BIOMEDICINES Volume12, Issue5 Article Number 998, https://www.mdpi.com/2227-9059/12/5/998, DOI10.3390/biomedicines12050998

M9. Normal Hypothalamic-Pituitary-Adrenal Axis by High-Dose Cosyntropin Testing in Patients With Abnormal Response to Low-Dose Cosyntropin Stimulation: A Retrospective Review By: Fleseriu, Maria, Gassner, Marika, Yedinak Christine, **Chicea Liana**, Delashaw Johnny B Jr., Loriaux, D. Lynn, ENDOCRINE PRACTICE Volume: 16, Issue: 1, Pages: 64-70, 1530-891X, DOI: 10.4158/EP09153.OR

M10. Studies to Elucidate the Mechanism of Cardio Protective and Hypotensive Activities of Anogeissus acuminata (Roxb. ex DC.) in Rodents, Saqib F, Arif Aslam M, Mujahid K, Marceanu L, Moga M, Ahmedah HT, **Chicea L**., Molecules. 2020; 25(15):3471. https://doi.org/10.3390/molecules25153471

M11. Biomolecular Screening of Pimpinella anisum L. for Antioxidant and Anticholinesterase Activity in Mice Brain., Mushtaq A, Habib F, Manea R, Anwar R, Gohar UF, Zia-Ul-Haq M, Ahmad M, Gavris CM, **Chicea L**. Molecules. 2023; 28(5):2217. https://doi.org/10.3390/molecules28052217

M12. Biomarkers for the Noninvasive Diagnosis of Endometriosis: State of the Art and Future Perspectives., Anastasiu CV, Moga MA, Elena Neculau A, Bălan A, Scârneciu I, Dragomir RM, Dull A-M, **Chicea L-M**. International Journal of Molecular Sciences. 2020; 21(5):1750. https://doi.org/10.3390/ijms21051750

M13. Ag Nanoparticles for Biomedical Applications—Synthesis and Characterization—A Review, Nicolae-Maranciuc A, Chicea D, **Chicea LM**. International Journal of Molecular Sciences. 2022; 23(10):5778. https://doi.org/10.3390/ijms23105778

M14. Comparative Synthesis of Silver Nanoparticles: Evaluation of Chemical Reduction Procedures, AFM and DLS Size Analysis. Chicea D, Nicolae-Maranciuc A, Doroshkevich AS, **Chicea LM**, Ozkendir OM. Materials. 2023; 16(15):5244. https://doi.org/10.3390/ma16155244

M15. Silver Nanoparticles-Chitosan Nanocomposites: A Comparative Study Regarding Different Chemical Syntheses Procedures and Their Antibacterial Effect, Chicea D, Nicolae-Maranciuc A, **Chicea LM**. Materials. 2024; 17(5):1113. https://doi.org/10.3390/ma17051113

M16. Results of light scattering dynamics analysis of biological fluids, D. Chicea, **L. M. Chicea**,
Journal of Optoelectronics and Advanced Materials Vol. 9, No. 3, March 2007, p. 694 – 697.

M17. The Application of AFM for Biological Samples Imaging, D. Chicea, B. Neamtu, R. Chicea, **L. M. Chicea**, Digest Journal of Nanomaterials and Biostructures Vol. 5, No 4, October-December 2010, p. 1015-1022

M18. HSA Particle Size Characterization By AFM, Dan Chicea, Radu Chicea, **Liana Maria Chicea**, Romanian Reports in Physics, Vol. 65, No. 1, P. 178–185, 2013.