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HABILITATION THESIS

Biomarkers, molecular mechanisms and therapeutic interventions in systemic inflammatory pathology

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The habilitation thesis, titled **"Biomarkers, molecular mechanisms and therapeutic interventions in systemic inflammatory pathology"** presents the synthesis of the research undertaken after obtaining the title of Doctor of Medicine. Currently, I hold the position of Associate Professor in the discipline of Medical Recovery (Physical and Rehabilitation Medicine) and Balneophysiokinetotherapy and recovery at the Faculty of Medicine of the "Vasile Goldiș" Western University of Arad. I am also a primary care physician in recovery, physical medicine and balneology, working within the Clinical Department of Recovery, Physical Medicine and Balneology of the Arad County Emergency Clinical Hospital.

The habilitation thesis is structured in three main sections:

SECTION I presents the evolution of my scientific, professional and academic career, being structured in eight chapters, as follows:

1) synthesis of scientific, professional and academic achievements;

2) cellular and molecular mechanisms involved in the inflammatory response and oxidative stress;

3) inflammatory and metabolic biomarkers in acute and critical pathology;

4) risk factors and immune response in infectious pathology;

5) microbiome, nutrition and systemic interactions in inflammation;

6) therapeutic response and personalized medicine in chronic inflammatory diseases

7) therapeutic interventions and pharmacological factors

8) clinical aspects and applications in systemic inflammatory pathology

The present paper represents a broad analysis of cellular and molecular mechanisms involved in the inflammatory response and oxidative stress, of inflammatory and metabolic biomarkers implicated in acute and critical pathology. We have highlighted the main risk factors and immune response in infectious pathology. In another context, the clinical aspects and applications in systemic inflammatory pathology, systemic interactions in inflammation, as well as the therapeutic response in chronic inflammatory diseases through therapeutic interventions and pharmacological factors were described. The research results emphasize the significant socio-economic impact of these inflammatory conditions and the need to develop advanced diagnostic and prevention strategies.

The first chapter of this habilitation thesis includes a detailed synthesis of scientific, professional and academic achievements, presenting both scientific contributions prior to obtaining the scientific title of Doctor of Medicine, and especially the achievements subsequent to obtaining this title.

In the second chapter, we addressed the impact of cellular and molecular mechanisms involved in the inflammatory response and oxidative stress. Therefore, the use of natural or synthetic antioxidants protects against oxidative stress caused by anthracycline drugs. Chrysin, garlic extract and silymarin are natural substances frequently used as dietary supplements, each with specific roles in supporting health, which have demonstrated efficacy in preventing doxorubicin-induced cardiotoxicity, while melatonin protects cardiac tissue against epirubicin-induced toxicity and L-carnitine against mitoxantrone-induced toxicity in mice, Chrysin 5,7-dihydroxyflavone (CHR) being a natural flavonoid and which is present in

large quantities in honey, propolis and many plant extracts. Thus, CHR exhibits a wide spectrum of pharmacological effects, such as antioxidant, antiallergic, anti-inflammatory and antitumor effects. At the same time, antiacyclins are among the most effective chemotherapeutic agents and remain important in the treatment of many malignancies, including breast cancer, sarcoma and lymphoma. Their use in clinical practice is limited by dose-dependent cardiotoxicity and, consequently, the use of lower doses may involve the risk of a reduced cancer response rate. Chysin attenuates mitoxantrone-induced cardiotoxicity, an important event in anthracycline-induced cardiac injury, with cardiomyocyte apoptosis playing an important role in drug-induced cardiomyopathy.

Previous studies have shown that chrysin ameliorates doxorubicin-induced cardiotoxicity by reducing oxidative stress, inflammation, and apoptosis, but the protective effect of chrysin on cardiomyocyte apoptosis and specific intermediate filament disruption triggered by mitoxantrone (MTX) has not been investigated.

Another relevant study was on the involvement of Genistein which enhances the cytotoxic, apoptotic, and oxidative stress-inducing properties of doxorubicin in SK-MEL-28 cancer cells, cutaneous melanoma representing the deadliest form of skin cancer and is the most common subtype of melanoma, over 90% of all diagnosed cases. The complexity and aggressiveness of the disease mainly result from its high level of heterogeneity and its increased metastatic potential, the development of cutaneous melanoma has been associated with both environmental and genetic risk factors. The main trigger for carcinogenesis being exposure to ultraviolet (UV) radiation from natural or artificial sources, and despite significant advances in understanding the genetics, biology and treatment of carcinoma, the curative options available for the management of this malignancy remain limited, especially in advanced stages of the disease. Currently, chemotherapy remains an option for the treatment of metastatic cutaneous melanoma, although no particular drug regimen has been found to provide an overall survival benefit to treated patients, the main impediments of current chemotherapies remaining the acquisition of multidrug resistance and various toxic effects. However, it has been well documented that the duration of response to targeted therapy is limited, and the clinical benefits after immunotherapy remain modest, as intrinsic or acquired resistance to these advanced tumor-targeted treatments have also been reported.

The next chapter of this habilitation thesis analyzes aspects of inflammatory and metabolic biomarkers in acute and critical pathology through studies on the evaluation of the role of TNF-alpha and IL-6 cytokines in the production of hypoalbuminemia in patients undergoing major surgery, the evaluation of plasma albumin as a potential prognostic biomarker in patients with traumatic SIRS, the evaluation of plasma levels of positive and negative hepatic acute phase proteins in patients undergoing major surgery, and the effect of red blood cell transfusion on oxygen supply and consumption in peripheral tissues in septic patients. The mechanisms that lead to a decrease in plasma albumin levels in patients with systemic inflammatory response syndrome (SIRS) caused by surgical trauma are represented by capillary wall permeability in conditions where hepatic albumin synthesis decreases, during the acute hepatic phase, albumin being considered a negative acute phase protein. At the same time, the study considered another possible mechanism of decreasing plasma albumin concentration - hemodilution, iatrogenic, induced by infusion solutions.

As for the permeability of the capillary wall, this phenomenon occurs under the simultaneous, direct and indirect action of several mediators, which can be divided into two groups:

- cellular mediators represented by peptide molecules with a proinflammatory role - TNF-alpha, IL 6, interleukin 8 (IL 8) and interleukin 1 (IL 1) which are secreted mainly by macrophages and are found in the interstitial tissue; the main role of these intercellular messengers is to stimulate the innate immune system to be able to react to SIRS. However, among all these cellular mediators, TNF-alpha is the most important mediator, since it has a prolonged action in the induced inflammatory response and also has the most important role in the synthesis of endothelial glycoproteins.

- plasma mediators are represented by the C5a complement fragments, bradykinin products of fibrinogen degradation; the main role is played by the C5a complement fragment, the activation of the complement cascade occurring in patients with SIRS secondary to surgical interventions (surgical trauma) through the lectin-mannose protein of positive reaction in the hepatic phase.

Albumin also has the role of modulator of vascular tone by binding and inhibiting nitric oxide (NO) produced by the vascular endothelium, as well as a possible anti-inflammatory role mainly due to the decrease in the interaction of the vascular endothelium with activated neutrophils.

Secondary physiological functions that are still the subject of numerous researches are represented by the antioxidant function, mainly due to free SH groups, approximately 80% of the plasma thiol interacting with oxygen reagents such as peroxide H2O2 and peroxynitrate (oNOo), annihilating them and forming the albumin sulfenic acid derivative, having a stabilizing role at the vascular endothelium level by reducing oxidative stress at this level. The acute liver response occurs as a result of tissue trauma, infection, or systemic inflammation. It is triggered in the liver by interleukin 1 (IL1) and interleukin 6 (IL6). This response has been shown to be the main mechanism for the decrease in plasma albumin levels in patients undergoing major surgery.

Another aspect described in the studies addressed was about the effect of red blood cell transfusion on oxygen delivery and consumption in peripheral tissues in septic patients. It is known that critically ill patients have endothelial dysfunction, which acts synergistically with the effects of blood storage to produce vasoconstriction. Before transfusion, the shape of red blood cells can be changed or their deformability decreases and they no longer allow easy access to the microvascularization, which leads to a decrease in oxygen supply to these areas. Sepsis is a major response of the body to infection and is a leading cause of death due to its progression to organ failure. The pathogenesis of organ dysfunction associated with sepsis also includes inadequate tissue perfusion and oxygen delivery. Patients with sepsis have changes in microvascular circulation, alterations in tissue oxygenation, oxygen metabolism, and increased lactic acid concentrations in the blood, all of which lead to the development of organ failure. To increase oxygen-carrying capacity, packed red blood cell transfusion is a viable treatment option.

Chapter 4 provides a description of studies on risk factors and immune response in infectious pathology, namely the evaluation of vitamin D deficiency as a risk factor in patients with moderate COVID-19, the analysis of the impact of SARS-CoV-2 infection on health, highlighting the complexity of the molecular mechanisms associated with this pathology, with the prediction of severe COVID-19 outcomes in the elderly: the role of systemic immune inflammation, liver function tests and the neutrophil to lymphocyte ratio. This research continued with another, namely a cross-sectional study involving retrospective serological assessment of vitamin D levels in children from western Romania.

The studies conducted provided an extended perspective on the long-term complications associated with the infection and significantly contributed to the understanding of the pathophysiological processes involved and to the identification of new pharmacological markers. The results obtained will open new horizons for future research and for the development of advanced therapeutic strategies. The review focuses on epidemiological characteristics, diagnostic methods and therapeutic strategies, highlighting their impact on public health.

In another study, we assessed the prevalence and antibiotic resistance of Streptococcus agalactiae in women of childbearing age with urinary tract infections. Urinary tract infections are among the most common bacterial infections, representing a public health problem, with women of childbearing age (15-44 years) having an increased vulnerability to urinary tract infections compared to men. Group B Streptococcus (GBS) infection causes significant morbidity and mortality worldwide each year, with the prevalence of invasive GBS infections being twice as high in pregnant women compared to non-pregnant women. GBS can also cause a range of severe invasive infections in vulnerable individuals, such as newborns, pregnant or postpartum women, the elderly, and individuals with compromised immune systems, diabetic patients, and those with pre-existing urological abnormalities. GBS can lead to bacteremia, pneumonia, osteoarthritis, skin infections, soft tissue infections, and urinary tract infections, including pyelonephritis and prostatitis.

The results contribute to the understanding of the regional epidemiology and treatment methods for this pathology, proposing integrated prevention and treatment measures in a local and global context.

Another important chapter (chapter 5) is that of the scientific approach to microbiome, nutrition, and systemic interactions in inflammation, with studies on the impact of the residual intestine after extensive intestinal resection on bacterial overpopulation and bacterial translocation. The aim was to demonstrate that, after extensive intestinal resection, there is a direct relationship between the number of intestinal bacteria (bacterial overload), abnormal morphological changes of the intestinal mucosa and BT to distal organs (MLNs, spleen and liver). We were also able to compare our findings with similar studies carried out in humans. The studies carried out revealed that a series of reactions were triggered that led to an increase in the number of bacteria (bacterial surface), abnormal morphological and functional changes of the intestinal mucosa and the translocation of bacteria to distant organs. Bacterial translocation in humans, although still unproven, is nevertheless assumed to be involved in multiorgan dysfunction syndrome, systemic inflammatory response syndrome, acute pancreatitis, cirrhosis, burns, ischemia-reperfusion syndrome and intestinal obstruction. The study was designed to compare the gut bacterial flora of cecal stools and duodenal fluid from rats with different degrees of intestinal resection (60%, 70%, and 75%) before and at the end of the experiment, and the rationale for this was to demonstrate that gut bacteria have an adverse effect on intestinal mucosal architecture and cell kinetics observed in histopathological analysis of the rat intestine. In recent years, the gut microbiota, a complex community of microorganisms that live in the human gastrointestinal tract, has emerged as a key player in human health and disease. This complex ecosystem, once considered a mere bystander in human physiology, is now recognized as an important module of diverse host processes, including metabolism, immunity, and even neurological function.

A systematic review of the role of short-chain fatty acids and the gut-retinal connection described aspects of retinal diseases, including age-related macular degeneration (AMD), diabetic retinopathy (DR), and glaucoma, which represent a significant global health burden and are collectively the leading causes of irreversible vision loss and blindness worldwide. AMD alone affects millions of individuals, predominantly

those over 60 years of age, indicating a substantial increase in prevalence in the coming decades. Similarly, diabetic retinopathy is a major complication of diabetes, affecting a considerable portion of the diabetic population and contributing significantly to vision impairment, and glaucoma causes progressive and irreversible damage to the optic nerve. Current therapeutic approaches for these diseases, such as anti-VEGF (vascular endothelial growth factor) therapy for neovascular AMD, complement inhibitors such as pegcetacoplan for dry AMD, laser photocoagulation for DR, and intraocular pressure-lowering drugs for glaucoma, have limitations. Although these treatments can slow disease progression and, in some cases, improve vision, they often fail to address the underlying causes of retinal diseases. This highlights the urgent need to better understand the pathophysiological mechanisms involved and to develop novel therapeutic strategies that target the underlying causes of these debilitating conditions.

The recognition of the profound influence of the gut microbiota on systemic health has stimulated research into its potential role in extra-intestinal organs, including the eye. This has led to the conceptualization of the "gut-retinal axis," a bidirectional communication pathway linking the gut microbiome to the retina. Recent studies suggest that imbalances in the gut microbiota, termed dysbiosis, may contribute to the pathogenesis of retinal diseases through several mechanisms. These include disruption of the integrity of the gut barrier, leading to increased translocation of bacterial products, such as lipopolysaccharide (LPS), into the circulation, thereby triggering systemic inflammation, which is known to affect retinal health.

Another important research direction has been studies that have particularly emphasized Therapeutic Response and Personalized Medicine in Chronic Inflammatory Diseases (Chapter 6). Here I participated in a multicenter study on histopathological aspects in hip osteoarthritis, OA being a degenerative disease of the mobile (synovial) joints that affects up to 70% of the elderly population. All diarthrose joints can be affected by OA, but the joints most commonly affected are the hand, hip, and knee. In the present study, since the pathogenesis of coxarthrosis is incompletely understood, we aimed to evaluate the histopathological changes (HP) that occur in hip osteoarthritis. From a pathological point of view, OA is characterized by complex lesions, such as: articular cartilage damage, subchondral bone changes, osteophyte formation, muscle changes and synovial tissue inflammation. In advanced stages of the pathological process, the histopathological picture highlights areas of poorly consolidated cartilage continuity solutions, accompanied by the proliferation of a fibrovascular granulation tissue, the presence of deep vertical fissures in the thickness of the cartilage, incongruities and marked deformations of the articular surfaces, extensive degradation of the extracellular matrix, as well as chondrocytic hypocellularity associated with degenerative morphological alterations.

In advanced stages of the pathological process, structural changes are defined by the presence of deep vertical fissures in the thickness of the cartilage, incongruities and marked deformations of the articular surfaces, extensive degradation of the extracellular matrix, as well as chondrocytic hypocellularity associated with degenerative morphological alterations.

Another elaborate study was related to the factors (especially age, sex, metabolic and pharmacological factors) that may influence the status of lack of response to therapies for rheumatoid arthritis. The pathogenic determinants involved in the establishment of primary non-response (PNRS) and secondary (SNRS) status in patients with rheumatoid arthritis (RA) include demographic and anthropometric variables such as age, sex and body mass index (BMI); however, the dynamics of these phenomena are

critically conditioned and differentiated by the selected pharmacotherapeutic profile and the duration of exposure to therapy. The chronic, progressive and recurrent inflammatory process at the articular level determines the degradation of hyaline cartilage, marginal bone erosion and, finally, severe alteration of the functionality of the distal extremities of the limbs. Although the onset of the pathology is frequently marked by an oligoarticular pattern, the advanced stages characteristically evolve towards global polyarticular involvement, being doubled by a complex extraarticular symptomatology.

Uncontrolled rheumatoid arthritis (RA) presents a severe clinical course, dominated by major systemic manifestations – with a predilection for the lungs, cardiovascular system and the hematogenous marrow – phenomena that directly correlate with a significant increase in mortality.

The suboptimal therapeutic response of RA patients to conventional biological agents and targeted synthetic therapies (targeted synthetic DMARDs) may be conditioned by a multitude of factors. Among these are the molecular inflammatory profile specific to each individual, polypragmatic drug interactions or the absence of rigorously defined biomarkers for the predictive assessment of efficacy, especially in the case of innovative molecules.

Conceptually, the absence of a favorable clinical response in the initial induction window defines the primary non-response status (PNRS). In contrast, secondary non-response status (SNRS) describes the progressive diminution or exhaustion of therapeutic efficacy after an initial clinical benefit has been achieved (5–7). Although these conceptual demarcations are unanimously accepted in the literature, there is still a lack of consensus regarding their standardization and implementation in current clinical practice.

An observational cohort study, namely Echocardiographic Follow-up in Outpatients with Systemic Arterial Hypertension, the main aim of our study was to evaluate longitudinal changes in echocardiographic parameters during 3 years of follow-up in a cohort of hypertensive patients receiving routine outpatient care. As a secondary objective, we examined whether the degree of blood pressure control, as assessed by annual 24-hour ambulatory monitoring, influenced the magnitude of these echocardiographic changes. In addition, we investigated possible associations between different classes and combinations of antihypertensive drugs and parameters of cardiac remodeling. Finally, we explored the impact of a supportive lifestyle.

The following chapter (7) addresses aspects of therapeutic interventions and pharmacological factors with applicability to a study on Thermal stability of piroxicam - Active substance and tablets I. Kinetic study of the active substance under non-isothermal conditions Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) used for inflammatory and painful diseases of rheumatic and non-rheumatic origin.

The anti-inflammatory activity of NSAIDs and most of their other pharmacological effects are related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are mediators of the inflammatory process. Thermal analysis is one of the most frequently used instrumental techniques, especially in areas of increasing importance, including the pharmaceutical field. Kinetic studies have become a crucial point in thermal analysis, where the main goal is to determine the mechanisms of the pyrolysis reaction. The aim of the present work was to evaluate the thermal behavior of piroxicam, active substance and tablets, respectively to determine the kinetic parameters for the active substance under non-isothermal conditions, as they represent a criterion for estimating its thermal stability.

Chapter 8 addresses clinical aspects and applications in systemic inflammatory pathology through a case study of a patient with esophageal ulcer associated with mild hemophilia A

Our aim was to highlight the link between coagulation deficiency (prolonged bleeding) and esophageal ulcer bleeding. We wanted to emphasize the difficulty of diagnosing mild hemophilia A, as well as the effectiveness of treatment (correction of plasma FVIII value) associated with endoscopic hemostasis. We also reviewed the wide range of possible clinical complications caused by severe hemorrhage and the treatment of delayed hemostasis. The association between peptic ulcer and hemophilia is extremely rare, only a few clinical cases have been reported.

SECTION II details the evolution and development plans of my career, both from the perspective of professional, scientific and academic activity, as well as new strategies proposed for the implementation of development directions.

On a professional and academic level, I aim to strengthen my mentor profile by assuming the role of doctoral supervisor, with the aim of guiding young researchers towards interdisciplinary approaches and high standards of ethics and excellence. From a scientific perspective, my priorities aim to expand the international visibility of my own research results by publishing in high-impact journals and by joining prestigious academic networks. To put these objectives into practice, the new proposed strategy will be based on attracting funds through competitive grants and on creating strategic partnerships between the academic and socio-economic environments.

Through this integrated approach, I aim to make an important contribution to increasing the prestige of the institution organizing doctoral studies (IOSUD) and to advancing knowledge in the field.

SECTION III includes the bibliographic references for all articles developed.

This habilitation thesis makes a significant contribution to deepening knowledge and optimizes the current framework for understanding major challenges in public health management. In this sense, the scientific core of the work focuses on the identification of new biomarkers with high predictive value and on the elucidation of those molecular mechanisms that govern systemic inflammatory pathology. By deciphering these complex signaling cascades, the research opens new perspectives for the development of personalized therapeutic interventions, capable of modulating the immune response and reducing the severity of clinical complications. By correlating regional and national specificities with international dynamics, the research goes beyond the theoretical sphere, providing working tools and practical solutions with applicability in a global context.