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PHD THESIS

THE NON-RESPONDER STATUS AND THE IDENTIFICATION OF NEW TISSUE BIOMARKERS WITH PROGNOSTIC AND THERAPEUTIC IMPACT IN AUTOIMMUNE JOINT PATHOLOGIES

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The main aim of this doctoral thesis was the clinical and experimental analysis of rheumatoid arthritis and, to a lesser extent, psoriatic arthritis through an interdisciplinary approach to these pathologies.

Rheumatoid arthritis, also known as RA, is a degenerative autoimmune disease that affects the joints over time. It is distinguished by a gradual and symmetrical inflammation of the joints that are affected, which eventually leads to cartilage breakdown, bone erosion and disability [1]. In the early stages of the disease, only a few joints are affected; however, as the disease progresses, the number of affected joints increases and extra-articular symptoms are common.

Rheumatoid arthritis is a chronic disease, which causes joint deformation and ankylosis, thus rheumatoid arthritis is not only a medical problem but also a social problem due to the fact that over 50% of patients stop their activity in the first 5 years of the disease, and 10% have a serious disability in the first two years of development. It is estimated that worldwide, the incidence of the disease is approximately 1.7% for women and 0.7% for men.

The chronic evolution of rheumatoid arthritis, with its disabling potential, involves a permanent evaluation, to assess the consequences of the illness and recovery. Establishing the clinical-evolutionary forms and stages of the disease, the active and remission periods, the degree of articular and systemic activity, the risk factors, prognosis and complications, requires multiple investigations and work tools, in repeated examinations. The prognosis, triple evaluated, for work, health and life, orients on the possibilities evolution of the disease. The early and certain diagnosis of rheumatoid arthritis triggers the monitoring of the evolution, prognosis and treatment to stop the inflammatory process and its consequences, in order to improve the quality of life of the rheumatoid patient and his family. The evolution in PAR is also important for the assessment of the social and personal costs of the disease, which consist of:

- direct costs (hospital and outpatient care);

- indirect costs (the inability to work of the patient with PAR and non-payment of the working days of those who take care of him);

- personal costs (reduced earning potential, shortened life expectancy, costs in terms of pain and disability);

- psychosocial costs (deterioration of the quality of life of patients, as well as of their families and friends).

An early diagnosis and one of certainty that occurs in chronic, progressive and disabling diseases requires a permanent monitoring of the evolution, prognosis and treatment, with the aim of stopping or at least stopping the progression of the inflammatory process and its consequences, in

order to improve the quality of life of patients with polyarthritis rheumatoid. Rheumatoid arthritis is the most common inflammatory rheumatism, this form of the disease affecting approximately 1 percent of the Romanian population, i.e. around 230,000 people. Improving the quality of life of patients with rheumatoid arthritis requires a social reinsertion and thus an increase in the economic and social standard of living.

Early stage RA is defined by widespread disease symptoms such as fatigue, a flu-like feeling, swollen and painful joints, and morning stiffness; it is accompanied by increased levels of C-reactive protein (CRP) and an increased erythrocyte sedimentation rate (ESR). The early stage of PAR is also marked by an elevated erythrocyte sedimentation rate (ESR). In contrast, rheumatoid arthritis that has not been adequately treated can present a complicated clinical picture, including the development of serious systemic manifestations such as pleural effusions, pulmonary nodules and interstitial lung disease, lymphomas, vasculitis of small or medium arteries, keratoconjunctivitis, atherosclerosis, hematological abnormalities (such as anemia, leukopenia, neutropenia) If considered as a whole, these systemic symptoms, which are caused by the persistent inflammatory state of patients with RA, cause an increased mortality rate. Although it is not known what causes RA, research [8] has shown that both genetic and environmental variables may play a role in the development of the disease. The initial establishment of PAR is likely to require two distinct events, as hypothesized for other autoimmune diseases: a genetic predisposition of the individual patient, which causes the generation of autoreactive T and B cells, and a triggering event, such as viral and bacterial infections or tissue damage, which provides activated antigen-presenting cells (APCs) to activate previously generated autoreactive lymphocytes, leading to the breakdown of tolerance.

In rheumatoid arthritis, the inflammatory condition known as synovitis affects the joint capsule, which includes the synovial membrane, synovial fluid, and the bones themselves. Dendritic cells (DCs), T cells, macrophages, B cells, neutrophils, fibroblasts, and osteoclasts play important roles in the initiation and maintenance of joint inflammation. Joint pain and swelling are symptoms of rheumatoid arthritis (RA), a condition characterized by persistent activation of the immune system in response to RA-specific autoantigens that are present in the joint and cannot be eliminated. Bone erosion and cartilage damage result from the persistent inflammatory environment of the arthritic joint, which causes pannus, an extension of the synovial membrane, to infiltrate the periarticular bone at the cartilage-bone interface. The ability of dendritic cells to capture, process and present antigens to naïve T cells is crucial for the regulation of immunological responses. In this context, the balance between the activation of the immune system and the induction and maintenance of tolerance is determined by the dendritic cell phenotype, which is characterized by the expression of surface molecules and the production of both cytokines and chemokines.

This suggests that the activated state of CD, together with increased production of pro-inflammatory cytokines, may encourage the presentation of autoantigens to T cells and the maintenance of inflammatory responses. DC activation in the inflammatory synovial microenvironment alters not only cytokine secretion patterns but also the expression pattern of, for example, chemokine receptors that govern DC movement. In rheumatoid arthritis (RA), for example, synovial DCs have been reported to express lower levels of CCR7, leading to low rates of emigration of mature DCs from inflamed tissues and persistence of local inflammation.

The prognosis of RA is considered to be influenced by three factors: positivity for rheumatoid factor (RF) and/or anti-citrullinated protein peptide antibodies (ACPA), early presence of structural lesions, and increased disease activity . Prognostic factors play a crucial role in the diagnosis of PAR, as well as in determining appropriate treatment options and estimating the severity of the disease. Currently, there is no standardized definition of poor prognostic factors. The main factors that are taken into account when making treatment decisions are increased disease activity, early presence of erosions and autoantibody positivity.

Adalimumab, Etanercept and Infliximab are potent neutralizing substances of the bioactivity of tumor necrosis factor alpha, which is the key cytokine in the pathogenesis of RA and APs, but there are fundamental differences between the three substances. Adalimumab is a humanized monoclonal antibody, Infliximab is a chimeric monoclonal antibody with a variable murine and human IgG1, Etanercept is a genetic fusion of a recombinant soluble p75 TNFalpha receptor and the Fc portion of human IgG. Etanercept binds to lymphotoxin, a cytokine found in synovial tissue. The complex formed between Etanercept and soluble and transmembrane TNF alpha is less stable than that formed with Infliximab. Infliximab and Etanercept proved effective in inducing apoptosis of macrophages but not lymphocytes in the synovial membrane.

Angiogenesis (pro-inflammatory) and lymphangiogenesis (anti-inflammatory) play an important role in modulating inflammation according to in vitro and in vivo studies demonstrated strictly on animal models.

Systemic autoimmune rheumatologic diseases, including connective tissue diseases, rheumatoid arthritis, and spondyloarthritis, have joint involvement along with extra-articular manifestations. Cutaneous disease frequently serves as the most significant and illustrative clinical presentation, as seen in conditions such as psoriatic arthritis, scleroderma, and systemic lupus erythematosus. In this particular context, it would be advantageous for rheumatologists to improve their understanding of skin conditions and their corresponding histopathological features. Examination of skin biopsy specimens can provide valuable assistance in verifying the diagnosis of typical and atypical clinical variations, as well as improving the understanding of the underlying pathogenic mechanisms and the strong correlation between skin and joint disorders.

As in most autoimmune diseases, the etio-pathogenic mechanisms of initiation and progression of rheumatoid arthritis and psoriatic arthritis are incompletely known and, moreover, the evolution and progression of the disease are unpredictable. The variability of etiopathogenic criteria determines an inconsistency in the establishment of therapy as well as in the response to first-line, secondary or biological therapy.

The most well-known skin lesion is the rheumatoid nodule. Other skin manifestations are poorly defined.

Migratory activated T cells interact with resident macrophages, dendritic cells, synoviocytes, and osteoclasts in the synovium. This is an area where many T-cell subsets may contribute to the pathogenesis of PR through complicated interactions [reviewed in [20].

T cells, B cells and monocytes are the predominant cells infiltrating the affected joints [7]. This process is initiated by synovial epithelial cells and continues with activated antigen presentation that primes autoantigen-specific T and B cell responses in local lymph nodes and organs.

Th1 cells, through the secretion of IL-2, IFN- and TNF-, strongly support other immune cells, the activation of macrophages and B cells and the initiation and maintenance of inflammatory reactions in the synovium [20 ,21,22] . Recent studies have shown that CD4+CD28null cells co-expressing perforin and granzymes, molecules more commonly found in CD8+ cytotoxic T cells, are increased in the peripheral blood of some RA patients [23,24,25] . These cells have been shown to play a role in helping the inflammation of PR. There is evidence that perforin-expressing CD4+ cells are present in the synovial fluid and tissue of patients with PAR [25,26,27]. These cells may have a role in both tissue injury and the maintenance of inflammation.

Skin biopsies are the easiest to obtain from a technical point of view, but still difficult in terms of the patient's agreement to do this. As this maneuver required an additional consent, this was only obtained from 30 of the 115 patients included in the study.

Even on a small number of biopsies, we demonstrated a variability of CLIC1 expression dependent or statistically significantly influenced by the type of therapy applied but also by other clinical-biological factors. Our results support the continuation of CLIC1 research in rheumatoid and psoriatic arthritis and the initiation of its quantification in the serum of patients with rheumatoid arthritis and psoriatic arthritis.

Data on CLIC1 expression in skin biopsies from patients are extremely rare and some are part of this study (141). The lack of CLIC1 expression data on human biopsies was also one of the motivations of the present study.

Intracellular chlorine channels are membrane-bound and participate in various pathological processes, including inflammation associated with neurodegenerative diseases, atherosclerosis, ankylosing spondylitis, tumor progression and metastasis of bladder cancer, renal cell carcinomas, or hepatocellular carcinomas. CLIC1 has a high capacity to translocate between the cytoplasmic and nuclear compartments depending on the functional state and based on this translocation the functions of CLIC1 are highly versatile. These versatile functions of chloride channels such as CLIC1 include the regulation of cellular metabolism, acting at the enzymatic level. The role of CLIC1 in inflammation, associated with various pathologies, is well known. In this context, CLIC1 is highly expressed in macrophages, the key cells of adaptive and innate immunity. CLIC1 expression is not limited to macrophages, but is also observed in connective tissue fibroblasts and myofibroblasts, which are abundantly found in various inflammatory lesions. The effects of CLIC1 on stromal cells are mediated by the presence of transforming growth factor beta (TGFβ) and tumor necrosis factor alpha (TNFα). In rheumatic diseases, CLIC1 is poorly studied, and experimental models mainly include macrophages or stromal fibroblasts isolated from mice.

The data presented above that underline the uncertainties regarding the evolution and response to therapy of rheumatoid arthritis and psoriatic arthritis represented the motivation to choose as a research topic the evaluation of the non-responder status in terms of the clinical part and, derived from this, the study type 1 chlorine channels, well known and described in inflammatory conditions but paradoxically little studied in rheumatoid arthritis and psoriatic arthritis.

Also, the release of interferon-gamma , interferon-beta, interleukin-18 and interleukin-23 by activated plasmacytoid dendritic cells (DDCp) has been associated with systemic inflammation in patients with rheumatoid arthritis (RA). Expression of anti-apoptotic B-cell activating factor (BAFF) by pDC can also stimulate the development of autoantibodies] . Consistent with this, the number of synovial pDCs in ACPA-seropositive RA patients is higher than that of ACPA-negative RA patients. RA patients also show increased levels of transcriptional activity in interferon- activated genes . Therefore, IFNs may play a significant role both in the onset of PAR and in its later, chronic, established phase.

So, the fact that cutaneous vasculitis is one of the first extra-articular clinical manifestations in RA and that skin biopsy is easier to perform and accepted by the patient, one of our hypotheses was to emphasize the importance of subclinical cutaneous vasculitis in the systemic outcome of patients with RA

Among the 115 patients included in the study, 50 patients agreed to have skin biopsies taken for the histopathological examination but also to start our study on CLIC1 expression in skin biopsies. These patients showed no unfavorable response to the administered therapies, whether they were conventional or biological therapies. As selection criteria, we collected skin biopsies from patients with clinical signs of vasculitis and hardly responsive to the applied therapies

There are three categories of rapunzels:

CLIC1 expression has been shown to be significantly associated with inflammasome activation, which facilitates the inflammatory response in rheumatic diseases. According to previous research (139), CLIC1 was observed to be expressed at high levels not only in stromal cells but also in epithelial cells, including endothelial and epidermal cells.

Using Jamovi statistical software, which is compatible with mac OS operating systems, we performed the statistical analysis of the collected data, as well as the construction of graphs, tables and correlation charts.

NRPs and NRSs are developed for anti-TNF and immune cell inhibitor therapeutic agents, specifically dependent on age, sex, BMI, and duration of treatment, but not for JAK inhibitors. Based on age, sex, and therapeutic classes, we identified female subgroups with an increased BMI from 40 to 59 years, a group that should be reevaluated in relation to the use of proinflammatory cytokine inhibitors. TNFα inhibitors induce NRS in all age groups, but according to BMI and sex.

Along with RITUXI, other pro-inflammatory cytokine inhibitors showed different age, BMI and gender interdependence with PRN or PRN status in the current study: (i) TOCI for induced PRN in patients with BMI>24.9 and (ii) Orencia for induced PRN in the same age, sex and BMI group as (i) but also for the group of women aged 60 to 81 with BMI<24.9 for both PRN and PRN. These results suggest that ORENCIA should be avoided in female patients aged 60-81 with a normal BMI.

In order to make therapeutic choices for rheumatoid arthritis (RA), prognostic variables have proven to be an effective clinical tool. Prognostic factors are important in three areas related to the treatment of early rheumatoid arthritis (RA): determining when to start treatment with disease-modifying antirheumatic drugs (DMARDs), deciding on the intensity of treatment, including changing therapies, and assessing the response to individual treatment [51].

TNF inhibitors induced NRS in both men and women in all three age groups and in both BMI subgroups. Whereas, for pro-inflammatory cytokine inhibitors, there are few and scattered data regarding the impact of BMI on their effectiveness, for TNF inhibitors there is already strong and relevant evidence that a high BMI reduced the effectiveness of the treatment and may induce a non- -answer [165,166]. In a study by Bergstra et al [166], the authors evaluated TNF inhibitors by stratifying patients into several BMI subcategories with more than 94% women. When they independently evaluated INFLIXI, ADA, and ETA, they found that patients classified as underweight based on BMI had an inconsistent response to INFLIXI and BA during the first year of follow-up. They did not refer to gender. Similar data were found for ADA and INFLIXI for BMI < 24.9 in the 22-39 age group, where the association between NRS status, ADA and INFLIXI was present. The doctors also said that INFLIXI and ETA have little therapeutic impact for obese patients, but did not provide details

about gender or age group. We found similar data for INFLIXI and ETA related to the development of NRS for these two drugs, but compared to the previously mentioned study, we highlighted these findings as specific to the group of men with a BMI>24.9, aged 40 and 59 years old. GOLI was associated with NRS for BMI>24.9 women aged 60 to 81 years, but we could find no data in the literature regarding the impact of BMI, age, or gender on response to GOLI therapy.

In a study by Magro et al (127), on a group of 43 patients, the authors described the predominant histological pattern, recognizing the presence of additional patterns of minor reactions in most cases. The predominant histopathologic manifestation observed in 21 subjects was characterized by palisade and/or diffuse interstitial granulomatous inflammation. The lesions showed a preference for the skin over the joints and included nodules, plaques, and papules. In addition to the presence of interstitial histiocytic infiltrates and variable collagen necrobiosis, these cases showed interstitial neutrophilia, vasculitis, and pauci-inflammatory vascular thrombosis. The primary morphology seen in another 11 patients was vasculopathic in nature. These included pauci-inflammatory vascular thrombosis, glomeruloid neovascularization, pustular, folliculocentric, leukocytoclastic, or benign cutaneous PAN-type neutrophilic vasculitis, granulomatous vasculitis, and lymphocytic vasculitis. In addition, occlusive intravascular histiocytosis have been identified, for which the proposed name is "RA-associated intravascular histiocytopathy". The prevalence of rheumatoid factor (RF) positivity and active arthritis was high among the investigated subjects. In addition, the presence of anti-Ro and anticardiolipin antibodies acted as competing factors, thereby exacerbating vascular damage in certain cases. The results of immunofluorescence testing performed on three patients indicated a predominant deposition of IgA in the vasculature. The clinical manifestations of urticarial plaques, pyoderma gangrenosum, and panniculitis in nine patients were mainly characterized by dermal and/or subcutaneous neutrophilic infiltrates.

Clinically, the symptoms of PAR differ dramatically between the early stages of the disease and the later stages of the disease that have not been adequately treated. Early stage RA is defined by widespread disease symptoms such as fatigue, a flu-like feeling, swollen and painful joints, and morning stiffness; it is accompanied by increased levels of C-reactive protein (CRP) and an increased erythrocyte sedimentation rate (ESR).

The early stage of PAR is also marked by an elevated erythrocyte sedimentation rate (ESR). In contrast, rheumatoid arthritis that has not been adequately treated can present a complicated clinical picture, including the development of serious systemic manifestations such as pleural effusions, pulmonary nodules and interstitial lung disease, lymphomas, vasculitis of small or medium arteries, keratoconjunctivitis, atherosclerosis, hematological abnormalities (such as anemia, leukopenia, neutropenia) If considered as a whole, these systemic symptoms, which are caused by the persistent inflammatory state of patients with RA, cause an increased mortality rate. Although it is not known what

causes RA, research [8] has shown that both genetic and environmental variables may play a role in the development of the disease.

It is likely that the initial establishment of PAR requires two distinct events, as hypothesized for other autoimmune diseases: (1) a genetic predisposition of the individual patient, which causes the generation of autoreactive T and B cells; and (2) a triggering event, such as viral and bacterial infections or tissue injury, that provides activated antigen-presenting cells (APCs) to activate previously generated autoreactive lymphocytes, resulting in the breaking of tolerance. Therefore, PAR is likely to develop in people who are genetically predisposed due to a combination of genetic variations, epigenetic changes, and environmental variables, which have been triggered by a random event (such as an accident or infection) [1]. Smoking, obesity, exposure to UV light, sex hormones, drug use, changes in gut, mouth and lung microbiota, periodontal disease (periodontitis) and infections have been identified as potential risk factors for the development of PAR [1,2,5,7 ,9,10]. The link between periodontal disorders and the development of RA is one of these risk factors that is particularly fascinating.

The initial batch included a number of 115 patients included in an Excel database over a 5-year period between 2017-2022. For the 115 patients, data were collected regarding age, sex, body mass index (BMI), types of therapy used, duration of therapy, response to therapy (responder, primary non-responder and secondary non-responder status. All these data have were collected in compliance with the GDPR laws, used and disseminated only after obtaining the patient's written consent regarding the ethical aspects of the research they were included in. Moreover, multiple organ damage, progressive, from rheumatoid arthritis and psoriatic arthritis is a factor of unfavorable long-term prognosis.

The manifestation of symptoms in patients with rheumatoid arthritis is influenced by various genetic factors, such as genetic susceptibility and epigenetic changes, as well as by environmental factors, such as smoking, obesity, and microbiome alterations in the oral and intestinal cavity regions. This results in a very diverse patient population.

These factors contribute to cellular immune proliferation and the formation of autoantibodies, which perpetuate the inflammatory cascade of the synovial membrane. Activation of multiple molecular inflammatory pathways in patients with rheumatoid arthritis may result in a unique treatment response for everyone [158].

- Primary non-responder: lack of response in the first 6 months after the initiation of biological therapy
- Primary secondary non-responder: primary response followed by failure to maintain positive effects, for a minimum of 12 months from initiation of treatment, or to achieve two positive scores within a minimum interval
- Late secondary non-responder: loss of positive response to biological therapy, after having more than 12 months of only positive effects to reach two positive scores in a minimum interval.

A study based on a meta-analysis was performed. Meta-analysis was chosen because it is a statistical tool that is able to collect a large amount of data, regardless of the type and heterogeneity of the study, even though we preferred randomized controlled clinical trials with similar design. To estimate the risk factor as vasculitis, meta-analysis is able to integrate all the data of individual studies and give us a grouping effect.

Ninety-two subjects in the studies included in the current meta-analysis were diagnosed with vasculitis and 706 patients did not meet the criteria for vasculitis. Regarding subclinical vasculitis, 92 patients from group one and 14 of 706 non-vasculitis patients presented with it. They developed a worse result. The overall effect was that subclinical vasculitis is a negative predictor for vasculitis (z: 1.86, p: 0.06)

There were no data on the involvement of CLIC1 in the inflammatory lesions of rheumatoid arthritis on human tissues from different regions affected by this pathology.

There is currently no specific biomarker for either rheumatoid arthritis or psoriatic arthritis, but only a panel of biomarkers, most of them non-specific, which can indicate the active status of the disease as well as the response to therapy.

One hundred and fifteen patients who were registered at the Rheumatology Department of the Târgu Mureș County Emergency Clinical Hospital were considered for inclusion in the study. The age of the patients varied between 22 and 81 years . During the course of this research, each patient was given information about the use of their data, while preserving their identity.

The main objective of the study was to emphasize the importance of the presence of (subclinical) vasculitis in the evolution of rheumatoid arthritis, an autoimmune, inflammatory disease. To achieve our objective, a meta-analysis was performed. Subclinical vasculitis attracts the attention of researchers after an imaging study that showed on a positron emission tomography (PET) aortic inflammation and such a connection with the cardiovascular manifestations of rheumatoid arthritis (RA). This is the breakpoint for linking the course of RA patients with subclinical vasculitis.

Patients were divided into three distinct age groups: group I includes patients aged 22 to 39 years, group II includes patients aged 40 to 59 years, and group III includes patients aged 60 to 88 years . This was done to investigate response to treatment and the relationship between response and body mass index, as well as duration of treatment. In a later part of the research, patients were assessed based on the type of treatment they received and their response to it.

People who are either overweight or obese, regardless of the degree of obesity, are known to have a high inflammatory state. As a result, the fourth classification of people in the research population was based on body mass index, first for people who were obese and those who were not fat, and then for the different forms of obesity based on BMI.

Rheumatoid arthritis patients who have failed an anti-TNF alpha biologic either due to ineffectiveness or toxicity are frequently switched to another biologic therapy on a 2nd anti-TNF agent. Patients with rheumatoid arthritis can be classified as primary or secondary non-responders, depending on the response to initial treatment. Baseline non-responders are those who have failed to respond both clinically and biologically, as demonstrated by lack of improvement in ESR and CRP levels.

This indicates that the drug is ineffective. Secondary non-responders, on the other hand, are patients who initially responded to treatment but later lost efficacy. The mechanism for primary and secondary non-responders may be different. Accurate identification of patients who have failed to respond to primary and secondary treatment is critical to improving patient care. Primary non-response is attributed to therapeutic failure, while secondary non-response is associated with immunogenicity-driven treatment failure. Non-response and development of antibodies are significant predictors of response to subsequent biologic therapy as indicated by some studies.

Providing accurate definitions of patients who have failed to respond to primary and secondary treatment is crucial for anticipating the most effective treatment for patients and improving clinical and practice guidance. It is less likely that patients with NRP are also NRS. There are also scattered data on the association between PNR and SPN status by age, sex, or BMI groups.

Rheumatoid arthritis patients show increased levels of CXCR5+ICOS+CD4+ T follicular helper cells in peripheral blood, and this increase correlates with both ACPA titers and disease severity. However, it is currently unknown how important they are for the pathogenesis of PAR.

PAR is one of the most common chronic inflammatory diseases, with a prevalence that varies from 0.4% to 1.3% of the population depending on factors such as gender (women are affected two to three times more often than men) , age (the frequency of new RA diagnoses peaks in the sixth decade of life), and patient cohort studies (the frequency of RA increases from south to north and is higher in urban than rural areas).

The pathogenesis of rheumatoid arthritis may be cytokine-specific and patient-dependent. The significance of a cell type may vary from patient to patient. The type of lymphocytes (B or T, Th1 or Th17) can affect the effectiveness of the therapy. These tools are applicable in the current clinical context for predicting the efficacy of biological therapy.

Numerous studies have evaluated the correlation between biologic therapies and factors such as age, sex, concomitant medication, and body mass index. Kleinert evaluated the efficacy of adalimumab by gender. Men are more likely to respond favorably to biologic therapy and achieve remission compared to women, study results show.

For the immunohistochemical technique, cases were stained by simple reactions using the following primary antibodies: mouse monoclonal anti human CLIC1.

The skin is one of the most frequently affected extra-articular organs in rheumatoid arthritis. Rheumatoid arthritis is frequently associated with the number of skin manifestations that can represent a warning about the severity of the disease. Rheumatoid nodules are the most common. Rheumatoid vasculitis, neutrophilic dermatoses as well. The skin is one of the organs that reacts the earliest to the autoimmune attack from the mentioned pathologies.

Rheumatoid arthritis is a systemic, autoimmune, inflammatory disease characterized by inflammation of the synovium at the joint level with clinical consequences represented by arthralgias at the level of the small joints of the hands and feet, swelling and accompanied in the absence of an adequate therapeutic management of bone loss corroborated with functional impotence. Synovitis is the hallmark of rheumatoid arthritis. Proliferated synovial or pannus is composed of cells that potentiate inflammation by secreting cytokines, chemokines that mediate angiogenesis. In acute inflammation, endothelial cells are activated by inflammatory mediators (TNFα – tumor necrosis factor, VEGF – vascular endothelial growth factor, interleukin 6 – IL-6, interleukin 1) leading to an increase in blood flow and thus to vasodilation, βedema due to the increase in vascular permeability. In chronic inflammation, blood vessels are dilated, hyperpermeable and activated due to the presence of adhesion molecules, leading to the continuous extravasation of inflammatory cells. The pannus is a vascularized structure, thus allowing the entry of effector cells responsible for joint destruction via autocrine and paracrine mechanisms. Joint swelling and pain are due to neovascularization, inflammatory cell infiltration with synovial hyperplasia, accompanied by an increase in the volume of the synovium and synovial fluid. Inhibition of neovascularization is one of the goals of RA therapy.

Rheumatoid arthritis is a chronic disease, which causes joint deformation and ankylosis, thus rheumatoid arthritis is not only a medical problem but also a social problem due to the fact that over 50% of patients stop their activity in the first 5 years of the disease, and 10% have a serious disability in the first two years of development. It is estimated that worldwide, the incidence of the disease is approximately 1.7% for women and 0.7% for men.

The prognosis of PR is thought to be influenced by three factors. According to source [52], there are three factors that indicate increased disease activity in rheumatoid arthritis: positivity for rheumatoid factor (RF) and/or anti-citrullinated protein peptide antibodies (ACPA), early presence of structural lesions, and increased disease activity .

Chlorine intracellular channel-associated protein 1 (CLIC1) is a constituent of the CLIC family and is considered one of the most highly conserved proteins. Its initial cloning was attributed to its increased expression in activated macrophages. The CLIC1 gene, also called NCC27, shows inconsistent and premature expression in human fetal tissues

Chlorine intracellular channel protein 1 (CLIC1) is part of the CLIC family and is one of the most conserved proteins. It was first cloned due to its increased expression in activated macrophages.

CLIC1, also known as NCC27, has early and variable expression in human fetal tissues and is found at low concentrations in human fetal brain but at high concentrations in human fetal lung, kidney, and liver. During adult human life, expression variability persists in most human tissues.

Proteins associated with chlorine channels have been intensively studied in inflammatory and malignant lesions, but not in autoimmune pathology. The role of chlorine channel protein 1 (CLIC1) was suggested in a single indirect article in an experimental model, describing the existence and activity of CLIC1 exosomal vesicles in rheumatoid arthritis inflammation.

The cytokines IL-12 and IL-23, which amplify antigen-specific Th17 responses, were found to be generated by mature DCs after extraction into the joint, leading to an unbalanced response pattern between Th1, Th2 and Th17 [31 ,32,33]. Inflammatory DCs (local differentiation from monocytes invading the inflamed joint) in the synovial fluid have been implicated in the pathogenesis of PR due to their ability to efficiently activate Th17 cells in the PR joints by producing TGF-, IL-1, IL-6 and IL-23 [14].

The cytokines IL-6, IL-1, IL-21, TGF- and IL-23 found in synovial joints have been shown to generate Th17 cells [31,32], which then recruit neutrophils, activate B cells and increase osteoclastogenesis [33,34]. However, the significance of IL-17A in PAR is controversial, as therapeutic targeting of IL-17A or IL-17R has shown less efficacy than, say, treating psoriasis [35,36]. Th17 cells may have immunosuppressive capabilities in PAR, as they have been found to produce IL-10 in response to anti-TNF treatment [37]. T1 cells that have developed into cytotoxic CD4+ T cells can cause both direct tissue damage and the production of pro-inflammatory cytokines [20], while T17 cells can be crucial in the early stages of the disease.

Membrane-bound intracellular chloride channels have been observed to play a role in various pathological processes, such as inflammation associated with neurodegenerative diseases (132), atherosclerosis (133), ankylosing spondylitis (134), tumor progression, and bladder cancer metastasis (135), renal cell carcinomas (136) and hepatocellular carcinomas (137). The possibility of CLIC1 translocation between cytoplasmic and nuclear compartments depends on its functional state.

Several specific markers of the lymphatic endothelium were identified: vascular endothelial growth factor receptor 3 (VEGFR-3), podoplanin, mucin-type transmembrane glycoprotein, propero-related homeobox 1, lymphatic hyaluronan receptor 1 (LYVE-1). It has been demonstrated that the VEGF family (VEGF C, VEGF D) affects lymphangiogenesis - "down regulating it", the pro-inflammatory cytokines TNFα and IL 1 stimulating the production of VEGF-C. The active pro-inflammatory role of TNF α and IL-1 in the pathogenesis of RA is known.

The goal of treating rheumatoid arthritis has evolved over the years from preventing symptoms, disability, and radiologic changes (joint destruction) to achieving early and sustained remission. Current

advantages can transform Rheumatoid Arthritis from a debilitating chronic disease into a curable pathology by stopping the autoimmune process and inducing immune tolerance. Immune disorders appear several years before the onset of arthritis. Autoimmunity is triggered in predisposed individuals with clinical manifestations of rheumatoid arthritis. Administration of appropriate treatment in the early stages of the disease can lead to sustained remission in rheumatoid arthritis (RA). This hypothesis suggests that treatment at the onset of the disease may have a greater potential to restore the autoimmune process and modify the course and phenotype of RA. Sustained remission is related to long-term disease improvement as measured by function, laboratory tests, improvement, and survival. Sustained remission can be achieved in both patients with early-stage disease and those with more advanced stages of rheumatoid arthritis [156].

The lymphatic system plays an important role in inflammation. Lymphatic circulation - the secondary vascular system, is known for its role in the removal of macromolecules, cells and fluids from the interstitial space and its function as a compensatory blood circulation system. Regulates the inflammatory response by transporting the synovial fluid, extravasated leukocytes and antibody-presenting cells to the lymph nodes and other secondary lymphatic organs, thus contributing to the decrease of edema-induced inflammation and the initiation of a specific immune response.

Unlike the data in the literature in which the non-responder status was evaluated on a wide age group, the current study aimed to study the primary and secondary non-responder status on age groups by stratifying the patients into 3 age groups . Until the present study, no significant differences were observed between primary and secondary non-responder status correlated with BMI. The stratification of patients by age groups demonstrated that BMI has a significant impact on the response to therapy depending on age. We also identified a variable response to pro-inflammatory cytokine inhibitors and TNF inhibitors, but not to JAK inhibitors, according to age groups.

The microscopic and immunohistochemical study involved the evaluation of 50 cases. The biopsy samples were obtained through minimally invasive surgical procedures. The study obtained the informed consent of the patients and the approval of the Research Ethics Commission

Our study of a cohort of 115 patients revealed large differences between the responses to different drugs, strictly related to age, sex, BMI or duration of treatment. Our statistical analysis included three classes of drugs used in the treatment of RA: anti-TNF agents (ADALIMUMAB, ETANERCEPT, GOLIMUMAB, INFLIXIMAB), JAK inhibitors (TOFACITINIB and BARITICINIB) and drugs that act on inflammatory cells (RITUXIMAB, TOCILIZUMAB and ORENCIA). We found that NRP or NRS were associated with anti-TNF and drugs that act only on inflammatory cells. But this association was highly dependent not only on age groups, but also on BMI status, sex, and duration of therapy.

Adipose tissue is a well-known additional source of TNF, adiponectin and inflammatory cytokines. It induces and promotes an inflammatory state that translates into a lower DAS28 score at the initial assessment of RA patients. Despite this, there are no clear data regarding its involvement in primary or secondary non-responses. Our data support the inclusion of adipose tissue as a factor in the poorer response to therapies targeting cytokine or TNF inhibition. Discrepancies between our results and other data in the literature may be due to the lack of stratification of patients based on age and also based on a differential assessment of patients according to BMI values (normal versus high BMI). Compared with other studies, our study assessed treatment response status for both overweight and obese patients, not only obese patients, as was done in most previously published studies.

All the structural components of the skin are affected by the autoimmune reaction induced by the variability of the autoimmune status and the inability of therapy to modulate this autoimmune reaction. The skin changes specific to the two pathologies are practically irreversible at a certain point in the evolution of the disease.

Recent studies have shown that subclinical inflammation (eg, the presence of Doppler signal within joints as seen on ultrasound) may be a marker for the outcome of RA patients. But, another minimally invasive study provided data that different types of synovitis may be related to RA outcome. Unfortunately, there are only a few studies with heterogeneous designs published in the literature, and to perform an intra-articular biopsy even if you are skilled in ultrasound (US) can be time- and resource-consuming and doctor- and patient-dependent [1].

A growing body of evidence suggests that alterations in the distribution and function of DCs contribute to autoimmune inflammation in PAR and other autoimmune disorders [reviewed in [11]]. It is likely that the increased migration of DCs to the inflamed joint [11] is responsible for the low frequency of conventional DCs and plasmacytoid DCs in the plasma of RA patients [12]. Increased expression of CCR6, the receptor for the chemokine CCL20, which is highly expressed in synovial tissue [13], was thought to be responsible for DC recruitment.

Eleven patients, representing 63.64% women and 36.36% men, were aged between 22 and 39 years . Of the seven female patients, three were identified as primary non-responders (NRPs), representing 42.85% of the female patient population. Conversely, all four male patients were identified as NRP, representing a 100% rate of NRP among the male patient population. Regarding secondary non-responder (NRS) status, the data indicated that 14.28% of patients were female and 75% were male.

The variability of response to conventional and biological therapy suggests the existence of factors specific to each patient and, for this reason, the initiation of personalized therapies for each individual patient is increasingly necessary to be taken into account.

Subclinical vasculitis attracts the attention of researchers after an imaging study that showed on a positron emission tomography (PET) aortic inflammation and such a connection with the cardiovascular manifestations of rheumatoid arthritis (RA). This is the breakpoint for linking the course of RA patients with subclinical vasculitis.

The present study is the first report on CLIC1 expression in rheumatoid nodules and the stromal and vascular compartments in skin biopsies from patients with RA and AP. Both diseases are characterized by inflammation involving multiple organs, including the skin, and it appears that CLIC1 is deeply involved. We demonstrated the expression of CLIC1 at the dermal level in rheumatoid and psoriatic arthritis in inflammatory skin lesions of RA and AP patients. We report here that therapeutic agents for RA and PsA can differentially influence dermal CLIC1 expression and its dynamics. Changes are independent of serum inflammatory markers. Therefore, we can conclude that the evaluation of CLIC1 in skin biopsies of patients with RA and APs can be used as a potential tool for predicting the general inflammatory state and response to therapy. Currently, there are no data on the serum evaluation of CLIC1 available for RA and PsA despite its function as one of the powerful factors in promoting inflammation. Our data strongly support the expanded evaluation of CLIC1 in RA as a potential novel tissue and serum marker in the assessment of inflammation and a predictive tool for response to therapy.