DOCTORAL THESIS
ABSTRACT

CLINICAL, HISTOPATOLOGICAL AND IMUNOHISTOCHEMICAL STUDY OF EARLY RHEUMATOID ARTHRITIS

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KEYWORDS: early rheumatoid arthritis, clinical, histopathological, immunohistochemical, arthroscopy, inflammation, synovial.
INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory systemic disease of unknown etiology that is characterized by persistent swelling and stiffness in joints and synovial joint destruction leading to severe disability with early mortality.

Long-term studies of rheumatoid arthritis show that most patients develop progressive disease with joint destruction radiologically visible, functional disability, reducing labor and significantly increasing mortality. Therefore, two concepts recently appeared in the treatment of rheumatoid arthritis - the ”window of opportunity” and ”tight control of disease.” This refers to a period of time in which there is a strong response to therapy, materialized through long-term benefits or even a "cure" of the disease. To identify as early as possible the disease, the term of “early rheumatoid arthritis” occurred. There is still a lack of prognostic factors able to guide optimal treatment adapted to each patient. From clinical perspective, if you enter an effective therapy before irreversible lesions develop, the prognosis could be improved. Economic consequences resulting from disease activity are decreased performance at work or inability to work.

The first histopathological studies of synovial membrane were based on biopsies obtained during surgery or postmortem studies. Arthroscopy is a minimally invasive technique for sampling synovial biopsies used successfully in rheumatology in the past 20 years. Examination of synovial tissue is used to investigate the histopathological changes, the etiology and the pathogenic mechanisms involved in rheumatoid arthritis. The study of cell populations or cytokines present in the synovia or in the serum of patients with RA can provide major details on early aspects of the disease and on the pathogenic mechanisms involved in RA activity.

Our study aimed to extend the knowledge of clinical, laboratory, histopathological and immunohistochemical factors involved in the production and progression of rheumatoid arthritis with the objective of identifying new prognostic markers and new therapeutic targets to stop as early as possible the disease progression.

GENERAL DATA CONCERNING RHEUMATOID ARTHRITIS

Arthritis is a term used to describe more than 100 different conditions that affect joints and other body parts. It is also one of the most common chronic diseases and one of the most common causes of disability in the population from all over the world.

The incidence is estimated at 95-150 new cases per 100,000 inhabitants per year [30]. Disease prevalence is approximately 1% of Caucasians, but varies between 0.1% to Africans in rural areas [179] and 5% for Pima Indians [42]. The disease affects about 8% of adults worldwide [63]. Disease sex ratio is about 3:1. The reasons for this superiority of women who develop disease are not clear, but it seems that genetic factors (X-link) and hormonal factors are involved [38]. However, men are more likely than women to die from extra-articular complications of rheumatoid disease [39]. Among the disease risk factors, patient age and gender, family history of disease and smoking are included.

The cause of rheumatoid arthritis remains unknown, being a worldwide very active research topic. It has been suggested that rheumatoid arthritis could be a manifestation of the host response to an infectious agent or a genetic susceptibility.

In clinical terms, rheumatoid arthritis is a chronic arthritis. In most cases, onset is slow, lasting weeks or months. In about two thirds of patients, it begins insidiously with fatigue, anorexia, generalized weakness and vague musculoskeletal symptoms until obvious
synovitis onset. Joint symptoms may be initially limited to one or several joints. Although the pattern of joint involvement may remain asymmetrical in few patients, the symmetrical pattern is more typical [149]. Rheumatoid arthritis affects synovial joints because the inflammatory process begins in the synovial membrane and then spreads to cartilage and bone. The main complaints of patients on presentation are related to these joints and those are pain, morning stiffness, swelling, or limitation of mobility and joint deformities. In rheumatoid arthritis, numerous extra-articular manifestations appear, especially in male patients with antinuclear antibodies (ANA) and positive rheumatoid factor (RF) [29].

The evolution of rheumatoid arthritis is quite variable and difficult to predict. Approximately 15% of patients with rheumatoid arthritis have an inflammatory process that is resolved in a short time, without major disability.

**MOTIVATION AND OBJECTIVES**

The study proposes a complete and thorough assessment of early rheumatoid arthritis with conventional methods of investigation (clinical and laboratory), as well as modern techniques such as immunohistochemistry and morphometry. Identification of complex mechanisms taking place at the molecular level and their interaction, provide valuable information regarding the occurrence and development of disease, and also on the assessment of prognostic in these patients.

This study evaluates the markers involved in different stages of the disease and aims to identify possible prognostic and therapeutic targets. Research in this direction is highly topical and achieving the required results can help to improve the quality of life in patients with this disabilitating condition.

The main specific objectives of the study include:
- Expanding knowledge of clinical, laboratory, imaging, histopathological, immunohistochemical and morphometric factors involved in the pathogenesis of the disease, in order to reveal its mechanisms;
- Identification and definition of morphological parameters that characterize early rheumatoid arthritis, for the purposes of early and differentiated treatment;
- Completion of morphological parameters for the assessment of early rheumatoid arthritis diagnosis by the possible molecular targets identified;
- Identification of mechanisms and markers involved in aggressiveness of early rheumatoid arthritis;
- Identifying the most specific markers of prognosis in early rheumatoid arthritis.

**MATERIALS AND METHODS**

The study is analytical, and the case selection was based on retrospective and prospective studies in which we compared the clinical, morphological and biomolecular diagnosis of 35 patients with early rheumatoid arthritis, from 2007 to 2010. For each patient we prepared a file of individual investigations in which we noted a number of parameters and the degree of its changes. The methods used in the study were clinical, paraclinical, imagistic, histopathological and immunohistochemical. Therefore the resulted data were statistically analyzed.
RESULTS

V.A. CLINICAL, PARACLINICAL AND IMAGISTIC STUDY IN EARLY RHEUMATOID ARTHRITIS

IV.A.1. Clinical study

Clinical trials aimed to collect data on all analyzed cases regarding age, sex, area of origin, time since illness onset, joint involvement, HAQ (Health Assessment Questionnaire) and DAS28 (Disease Activity Score) values.

With regard to patient age, between 51 and 60 years were most of them. We found a greater number of women affected by the disease, accounting for 24 of these 35 cases, representing 68.57% of the studied casuistry. The average was 10.57 ± 6.05SD painful joints / patient and 8.85 ± 5.43 SD swollen joints / patient. The majority, represented by 18 cases (51.43%) had a DAS28 score of over 5.1 with these values supporting high disease activity. HAQ values ranged between 0.7 and 2.9. We found moderate disability in 16 patients, representing 45.71% of the patients studied.

IV.A.2. Paraclinical study

The study included the investigation of values obtained in some biological laboratory tests with significance in disease progression, such as C-reactive protein (CRP), rheumatoid factor (RF) and anti-citrullinated proteins (anti-CCP) antibodies.

Values of CRP titers recorded in patients examined by us were between 1.14 and 122 mg / l, with an average value of 33.86±34.24SD mg / l. The values of RF titers in patients examined showed values between 8.5 and 42.3 IU / ml, with a mean value of 22.06±9.8SD IU / ml. Anti-CCP values were between 10 and 286 EU with an average of 102.0±87.05SD.

IV.A.3. Imagistic study

When performing knee arthroscopy in early RA cases, we examined the appearance of synovial membrane, articular cartilage and synovial fluid, focusing particularly on the changes of the synovial membrane. We noticed a synovial hypertrophy in 23 patients, representing cases of intense synovial cell proliferation. Depending on the degree of synovial hypertrophy, could see granulations, burgeons or synovial villi. Of the analyzed cases, we found synovia microcirculation intensely congested, with small caliber vessels dilated in 21 patients.

V.B. HISTOPATHOLOGICAL STUDY OF SYNOVIAL MEMBRANE IN EARLY RHEUMATOID ARTHRITIS

Histopathological study of the 35 cases of rheumatoid arthritis, aimed the assessment of synovial cell proliferation severity, inflammatory infiltrate, fibroblast proliferation, fibrinoid necrosis and vascular changes.

- Depending on the intensity of synovial cell proliferation in the investigated cases, we found grade 1 proliferation in 5 cases (14.3%), grade 2 in 9 cases (25.7%), grade 3 in 12 cases (34.3%) grade 4 in 9 cases (25.7%).
- Depending on the intensity of inflammatory cell infiltrate, we met as many degrees of severity as for synovial cell proliferation - grade 1 in 19 cases (54.3%), grade 2 in 10 cases (28.6%), grade 3 in 4 cases (11 , 4%), grade 4 in 2 cases (5.7%).
- Analyzing the studied cases in terms of the extent of fibrinoid necrosis we found that they corresponded in order of frequency to: grade 0 in 11 cases (31.4%), grade 1 in 9 cases (25.7%), grade 2 in 8 cases (22.8%), grade 3 in 7 cases (20%).

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• Analysis of the fibroblast proliferation intensity indicated the following: grade 0 in 6 cases (17.1%), grade 1 in 13 cases (37.1%), grade 2 in 9 cases (25.7%), grade 3 in 7 cases (20%).
• For the cases analyzed we have quantified the extent of vascular changes depending on the presence of one or more associated changes, such as congestion, increased vessel number that are located superficially, under the synovial cell lining layer, erythrocyte extravasation, proliferation of endothelium of arterioles and postcapillary venules.

Degrees given for each morphological change was necessary to achieve a composite histological score.

V.C. STUDIUL IMUNOHISTOCHIMIC AL INFLAMAȚIEI ÎN SINOVIA REUMATOIDĂ

Of the 35 cases of early rheumatoid arthritis investigated in a time period of 4 years (2007-2010), were selected for immunohistochemical study a total of 24 cases.

V.C.1. Study of inflammatory cells involvement in early rheumatoid arthritis

The study of CD20cy immunostaining was positive, whatever the extent of inflammatory infiltrate, the disposition being diffuse perivascular or follicular.

The study of CD45R0 immunostaining was also positive in all cases, the T cell staining being a cytoplasmic one. The extent of CD45R0 stained cell infiltrate, mostly corresponded with grade 2 of severity (58.3%).

We observed the presence of CD68 immunostaining with increased intensity both in lining membrane synovial cells and under lining ones, associated to inflammatory infiltrate.

V.C.2. Study of cytokines involvement in early rheumatoid arthritis

Immunoreaction for leptin and leptin-receptor was present in only 10 and 12 respectively of the 24 cases investigated. Positivity for leptin and leptin-receptor corresponded to cases with moderate and severe inflammatory infiltrate, grade 2 and 3 respectively. Immunoreaction for visfatin was present in only 15 of the 24 cases investigated. Reactivity investigation to IL17 of synovial tissue in patients with early rheumatoid arthritis showed immunostaining presence in 6 cases (25%).

V.C.3. Study of citrullinated proteins involvement in early rheumatoid arthritis

Citrullination was present both intracellular and extracellular. The results of this study indicate that intracellular immunoreactivity to citrullinated proteins is present in the synovia of patients with rheumatoid arthritis.

We observed positivity in lining synovial cells and in the deep ones. Positivity varied from isolated cells to compact cell groups, regardless of their degree of proliferation. Also, for citrullinated proteins were positive endothelial cells, fibroblasts and mononuclear cells from the inflammatory infiltrate.

V.D. STATISTICAL DATA ANALISYS

We tried to see the relationship between clinical parameters of disease activity (DAS28 and HAQ), a marker of inflammation (CRP) and those of autoimmunity (anti-CCP, RF), ANOVA test (analysis of variance) for comparing the results of clinical evaluation indicates highly significant differences (VHS) (p <0.001) between the analyzed parameters. We also noticed the correlation between clinical and pathological evaluation in arthroscopic synovial vasculature and histopathological evaluation of it by a significant degree of
correlation (p < 0.05). We obtained very high significant correlation of synovial cells proliferation with DAS28, synovial hypertrophy and macroscopic vascularity increase, of the inflammatory infiltrate with CRP, of the proliferation of fibroblasts with DAS28 and anti-CCP antibody titers, and of the microscopic vascular degree with DAS28 and anti-CCP antibody titers.

Chi square test of the parameters evaluated in the immunohistochemical study revealed a very high significant correlation (p < 0.001) of leptin receptor only with CD45R0 and visfatin. We also found a highly significant correlation (p < 0.01) of the leptin receptor with IL-17, and leptin with CD45R0, visfatin and IL-17. To identify T and B cells and to achieve a ratio between the two cell types we used CD45R0 and CD20cy immunostaining. Pearson test showed a positive linear distribution of T and B cells (Pearson index = 0.98). Regarding the relationship between immunohistochemical study data with clinical parameters, it is obvious the highly significant correlation (p < 0.01) of DAS28 score with leptin and its receptor, indicating an increase in leptin and its receptor during periods of high disease activity.

VII. CONCLUSIONS

This study comprising 35 cases of early rheumatoid arthritis selected from 2007 to 2010, allowed the following conclusions:

- Clinical study showed that most cases of early rheumatoid arthritis were diagnosed in the age range between the V and VI decades of life (88.6%) and effect on ratio by sex was for females, with 68.6% of the cases studied. Among patients, the majority (23 cases) had fewer than 10 affected joints. After HAQ values, 45.71% of patients had moderate disability and 37.14% had severe disability. DAS28 score values reflected a high disease activity.

- Paraclinical study included investigation of values obtained in some laboratory tests with biological significance in the progression of disease such as investigation of anti-CCP combined with RF and CRP, these helping to an early and accurate disease detection. Arthroscopy allows grading of visible macroscopic synovial mass. Synovial biopsy is particularly useful in cases where routine evaluation did not put the diagnostic and in patients detected in the early stages of the disease.

- Histopathological study of the synovial fragments obtained by targeted biopsy in patients with early rheumatoid arthritis can provide valuable guidance on disease severity. Characteristic morphological changes but not disease-specific, some of them almost every time present, are: synovial cell proliferation, inflammatory infiltration, fibrinoid necrosis, proliferation of fibroblasts and vascular changes;

- Statistical study analyzed the relation between all the evaluated parameters. The study of histopathological parameters correlation with other investigated parameters revealed a very high significant correlation (p < 0.001).

- Immunohistochemical study showed the involvement in RA pathogenesis of both types of lymphocytes, developed lesions being a consequence of activation and cooperation of the two cellular population types. Close distribution of B and T cells in the inflammatory infiltrate in rheumatoid arthritis shows interdependent pathogenic mechanisms. Chi square test showed a very high significant correlation (p < 0.001) with synovial vasculature. Regarding the location of CD20+ B cells, we noted that they had in most cases a direct relationship with neoformation vessels. Chi square test showed very high significant correlation (p <0.001) with inflammatory infiltrate and synovial vasculature. In cases with severe inflammatory infiltration, CD45RO+ T cells predominated in the external areas of lymphoid infiltrate. Within the lymphoid infiltrate areas is a small number of CD20+ B cells. Coexpression of leptin and its receptor in synovial cells, synovial fibroblasts and
inflammatory cells confirms the involvement of both autocrine and paracrine control loops in the pathogenesis of early rheumatoid arthritis. Chi square test showed very high significant correlation (p <0.001) of leptin and leptin receptor with inflammatory infiltrate and DAS28.

The presence of visfatin imunoexpression in synovial structures of early rheumatoid arthritis patients, suggests its role in modulating inflammatory environment in these patients. Positive IL-17 even in a small number of cases is an argument for its pathogenic participation in early rheumatoid arthritis.

Citrulline, positive in 87.5% of cases under study, may represent a new diagnostic possibility in order to distinguish, on biopsy fragments, the rheumatoid arthritis from other synovial inflammatory diseases.

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Self-assessment  

European level (*)

French  
C1 Proficient user

English  
C1 Proficient user

Driving licence(s)  
B

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