

*Evaluation of microcirculation by nailfold videocapilaroscopy in patients with rheumatoid arthritis - correlations
with clinical and immunological profile and markers of disease activity*

UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA

DOCTORAL SCHOOL

PhD THESIS

- SUMMARY -

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EVALUATION OF MICROCIRCULATION BY NAILFOLD
VIDEOCAPILAROSCOPY IN PATIENTS WITH RHEUMATOID ARTHRITIS -
CORRELATIONS WITH CLINICAL AND IMMUNOLOGICAL PROFILE AND
MARKERS OF DISEASE ACTIVITY

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SUMMARY

Rheumatoid arthritis (RA) is a systemic, autoimmune disease characterized by persistent synovitis, affecting small joints of the hands and feet. The disease is immunologically defined by specific immunomarkers - rheumatoid factor (RF) and citrulline cyclic anti-peptide (ACPA) antibodies.

It is one of the most disabling diseases and the most common form of arthritis affecting 0.5-1% of the population.

The etiopathogenicity of the disease is multifactorial and influenced by genetic, environmental, hormonal and immunological factors. The onset of pathogenic cascade occurs many years before the clinical onset and involves a specific genotype associated with loss of immune tolerance and self-reactivity. Proinflammatory cytokines have a central pathogenic role. TNF- α is fundamental in the synthesis of cytokines and chemoattractants, expression of adhesion molecules, promotion of angiogenesis, and suppression of Treg lymphocytes. IL-6 promotes leukocyte activation, anemia, autoantibody and acute phase reactants synthesis. IL-1 expressed in the synovium and promotes activation of leukocyte, endothelial cells, chondrocytes and osteoclasts. IL-17 and 17A activate FL synoviocytes, chondrocytes and osteoclasts as promoters of structural articular matrix degradation. JAK kinases responsible for the intracellular transmission of the cytokine-receptor binding signal play a significant role in the pathogenic cascade.

The interaction between environmental and the genetic factors is the starting point for pathogenic mechanisms, both components of this interaction being necessary but not sufficient independently. One example is the consistency rates of monozygotic twins - 10-15%, compared to the prevalence of about 1% in the general population. The "susceptibility epitope" at DR4 β with the strongest association is DRB * 0401, DRB * 0404, DRB * 0101, and DRB * 1402. The HLA-DRB1 haplotype is known to have the most genetic risk. Non-HLA genes - unineucleotide genetic polymorphisms at promoter regions, genes encoding proinflammatory cytokines, non-cytokine genes (PADI, PTPN22, etc.) are also involved. Associations have been described in relation to genes encoding for TNF α . Among the non-cytokine genes, PADI (peptidyl arginase deiminase) and PTPN22 present the most significant susceptibility risk.

Exposure to cigarette smoke and silicon powders appears to have an unfavorable effect in terms of susceptibility. The use of oral contraceptives seems to have a modest protective role,

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probably in relation to hormonal profile change. The hypotheses of the participation of viral and bacterial antigens in the initiation of pathogenic mechanisms are also considered. One of the mechanisms involves direct action of the pathogen by infecting the synovium and triggering a local inflammatory response that self-perpetuates.

The idea that one of the specific pathogenic elements is represented by the aberrant immune response against self-antigens was confirmed by the identification of RF and ACPA.

Although RA is a disease of the synovial joints, the systemic disease is evident and can result in a variety of manifestations - skin (vasculitis, rheumatoid nodules), cardiac (pericarditis, cardiomyopathy, valvulopathy, coronary vasculitis, etc.), pleuro-pulmonary (interstitial lung disease, pulmonary arterial hypertension, pleurisy, nodular pulmonary disease, bronchiolitis, etc.), kidneys (secondary amyloidosis, necrotizing focal glomerulopathy, etc.) and eye (episcleritis, scleritis, scleromalacia perforans).

Early diagnosis and therapy represent the main principles of disease management, the result of applying these two principles, materializing in achievement of remission or improvement of structural progression.

Novel assessment methods allowed the identification of unfavorable prognostic factors as well as parameters for monitoring the response to treatment. In current practice, rheumatologists use a set of clinico-paraclinical variables such as joint pain, tender and swollen joint count, disease activity assessed by physician and patient, ESR and CRP, specific immunomarkers, imaging parameters – radiographic and/or ultrasonographic. ESR and CRP are extremely useful for assessing disease activity as an integral part of DAS 28 (4v) and SDAI scores. Evaluation of RF and ACPA is of particular importance. Imaging evaluation includes three techniques - hand and foot radiography is the most commonly used method that allows erosion detection, articular ultrasound has utility in identifying synovitis and erosions and magnetic resonance has the benefits of a high-performance examination that allows identification of early lesions.

From the moment of diagnosis, patients with RA require regular monitoring to achieve and maintain favorable outcomes. Close monitoring is important for disease activity, structural progression and treatment side effects assessment.

Patients with RA have an increased cardiovascular risk compared to the general population comparable to patients with diabetes, a risk increased not only by classical factors, specific ones - ex.

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systemic inflammation - being an important element in the installation and perpetuation of endothelial dysfunction and subsequent atherogenesis.

Particular association RA - micro/macrovacular damage with fatal repercussions required the publication of a set of recommendations for evaluation, identification and management of patients with RA from the perspective of cardiovascular risk.

Cardiovascular mortality and morbidity is the main consequence of accelerated atherogenesis secondary to endothelial dysfunction. Persistent systemic inflammation and the relationship between inflammation-traditional cardiovascular risk factors appear to play a major role in the occurrence and perpetuation of atherosclerosis by direct and indirect effects on endothelium. The optimal control of the traditional risk factors is imperative but probably insufficient, strict control of systemic inflammation being necessary.

Capilloscopy is a reliable, non-invasive technique available to identify microvascular anomalies frequently encountered in rheumatic diseases. The main indication for capilloscopic assessment is Raynaud phenomenon. Beyond its primary indication, capilloscopy provides valuable information on the evolution possibilities of scleroderma-like disorders, the capilloscopic patterns identified being correlated with disease activity and organ damage.

In RA, the utility of capilloscopy for identifying possible correlations between microvascular anomalies and clinical profile, immunological status and disease activity is still insufficiently studied.

Although information on PR capilloscopy is very limited, it dates back to the 1980s when Merlen and Sarteel describe three capilloscopic anomalies associated with RA, namely multiple dilated capillaries, elongated capillaries, visibility of subpapillary venous plexus, megacapillaries, hemorrhages and tortuous capillaries. The authors place these microvascular alterations in different prognostic groups suggesting the existence of a typical rheumatoid microangiopathy.

Dervet studies microvascular alterations in 80 patients with PR and identifies a reduction in capillary flux - "sludge" - in 74%, neoangiogenesis in 59% and spontaneous haemorrhage in 28% of cases, suggesting the possibility of using capilloscopy as a tool disease monitoring.

McGill and Gow studied, on 30 patients, the ability to discriminate microvascular alterations identified in scleroderma (10 pts), SLE (9 pts) and RA (11 pts). Although the study group was small, the authors conclude that capillaroscopy has a specificity of 89% and a sensitivity of 80%, suggesting that the technique can distinguish with significant accuracy, the patients with scleroderma from those with SLE and RA.

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Altomonte studies, on 32 RA patients, microvascular alterations and frequently identifies elongated, tortuous capillaries and subpapillary venous plexus (in patients with positive ANA and RANA). It concludes that elongation and capillary tortuosity are the major changes in PR patients and that the visibility of the subpapillary plexus could be the expression of endothelial dysfunction induced by the presence of RANA in the vascular walls.

Kuryliszyn-Moskal studies the correlation between sICAM-1 and microvascular alterations in 79 RA patients and rheumatoid vasculitis versus 30 control. Identifies significantly higher values of sICAM-1 in RA versus control patients and in those with vasculitis vs. RA and a insignificant correlation between sICAM-1 values and capilloscopic pattern, although 75% of patients with severe microvascular changes exhibited values above normal of sICAM-1. The sICAM-1 values correlated with inflammation (ESR) and did not correlate with the duration of the disease, radiological status, age or treatment. It concludes that increased serum levels of sICAM-1 reflect rather a systemic vascular interest than local endothelial activation.

Pache M tests the hypothesis of ET-1 involvement of endothelial dysfunction in 12 RA patients by measuring ET-1 levels and capilloscopy (with cold test). The results highlight significantly elevated serum levels of ET-1, diminished capillary blood flow and pronounced vascular spasm after exposure to low temperatures in 58% of patients with RA.

Errichetti examines by capillaoscopy 15 patients with psoriatic arthritis without psoriasis, 12 RA, and 12 control patients and studies differences in microvascular pattern in order to identify distinct patterns associated with the two entities whose clinical differentiation is sometimes difficult. Identifies short, tortuous capillaries, almost perpendicular to the skin in psoriatic arthritis patients without psoriasis, and elongated capillaries, parallel to skin and irregular capillaries in patients with RA. It concludes that videocapillaroscopy may represent a differential diagnosis tool in patients with psoriatic arthritis without psoriasis or RA.

A larger study of patients – 201 RA and 50 control - developed by Sag S, aims to identify correlations between microvascular alterations, disease activity, and demographic characteristics in RA patients. Finds that approximative half of the patients had a nonspecific capillaroscopic pattern and a greater incidence of dilated and branched capillaries compared to the control group, a reduced correlation between tortuosity and disease duration and does not identify any correlation between microvascular alterations and immunological parameters - RF, ACPA - inflammation - CRP - or disease activity (DAS28).

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Therefore, we can conclude that the hypothesis of a specific RA capillaroscopic pattern has no support, and regarding the correlations between microvascular alterations and disease parameters the information in the literature is far too limited to allow for appropriate conclusions.

The aim of the research is to extend the knowledge about RA by studying microvascular anomalies as well as to establish correlations between identified capilloscopic changes and clinical, biochemical, immunological, imagistic and functional factors.

The prospective study, included 51 patients, 45 women (88.23%, mean age 46.13 ± 10.98 years) and 6 males (11.76%, mean age 46.67 ± 7.94 years) with RA (ACR / EULAR 2010 Criteria); study protocol was endorsed by the Ethics Commission of Craiova University of Medicine and Pharmacy.

The individual variables collected from each patient consisted of:

1. demographic data - age, sex
2. personal medical history - co-morbidities and their current treatment, past illness
3. history of joint disease (RA) - onset, diagnosis, treatment. The definition of precocity (early RA) was established as less than or equal to 6 months after the onset of symptoms.
4. risk behaviors - smoker / non-smoking status, alcohol consumption, drugs
5. relevant clinical data - results from the general clinical examination
6. specific rheumatologic evaluation - morning stiffness, TJC and SJC, VAS pain and VAS disease activity - patient and physician appreciation, HAQ-DI questionnaire, DAS 28 (4v) -CRP, DAS 28 (4v) -VSH, CDAI, SDAI
7. biological samples - hemoleucogram, aminotransferases, serum creatinine, serum glucose, ESR, CRP, RF, ACPA, ANA, urine exam.
8. Radiographs of hands and forefoot
9. postero-anterior and, where appropriate, lateral incidence chest X- ray
10. abdominal-pelvic ultrasonography
11. Doppler 2D cardiac ultrasound
12. electrocardiogram
13. nailfold capilloscopy.

The capilloscopic study was performed in each patient at the Center for Rheumatology Research, Department of Rheumatology, University of Medicine and Pharmacy Craiova using

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Videocap 3.0 (DS Medica, Italy) and 200X camera. Each capilloscopic parameter was quantified according to current standards.

For statistical processing, Microsoft Excel programs were used together with the XLSTAT suite for MS Excel and IBM SPSS Statistics 20.0.

17 patients (33.33%) had early RA - 16 women and one male - and 34 (66.66%) had established disease - 34 women and 5 males.

All patients with established RA had erosions and only 4 patients (23.52%) with early RA. Considering the potential of conventional radiography, in terms of erosion identification in patients with early disease, we can conclude that the percentage (25.32%) is not the real situation of erosive status in early RA patients.

3 patients (~ 6%) were in remission, 8 (~ 16%) had low-activity disease, 16 (~ 31%) had moderate disease activity and 24 (~ 47%) had high disease activity. Approximative 78% of the patients studied had moderate or high active disease. Although therapeutic management and the pharmacological arsenal had significantly changed, most patients with RA do not achieve remission or low disease activity.

Regarding immune status (RF, ACPA) we noticed a higher incidence of positive ACPA compared to RF. RF positivity (RF > 14 U/L) was identified in 74.5% of patients and ACPA positivity in 86.27% of patients. The percentages are similar to those in literature - RF can be identified in approximately 90% of the patients with RA and ACPA in 55-91%. Double serology - RF and ACPA positivity - was found in a significant percentage of patients - 60.78%. ANA positivity (low titer - 1/100) was found in 5.88% of patients - a common finding in clinical practice.

Only two patients had extraarticular disease - 1 patient with rheumatoid nodules and 1 patient with pericarditis. Both were female, had erosions, active disease and high titers of RF. Given the low number of cases with extraarticular manifestations, there was no possibility of including them in statistical processing. No case with rheumatoid vasculitis or Raynaud phenomenon has been reported. Incidentally, extra-articular manifestations are, at this time, much less commonly found in patients with rheumatoid arthritis.

The most frequent capilloscopic changes were the elongated capillaries (86.27%), tortuosity (76.47%) and subpapillary venous plexus visibility (94%). Dilated capillaries were identified in 17.64% of patients. Over 75% of the patients expressed this type of capillaroscopic changes. In contrast, none of the patients had haemorrhage, avascular areas, bushy or giant capillaries. Capillary

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density was preserved in the majority of patients – only 11.76% of patients had a slightly reduced density (7-9 capillaries per linear mm). All patients showed a non-specific capilloscopic pattern.

Disease duration was significantly correlated with capillary length (p Chi = 0.002). In a small number of patients - 7 (21%) - with established disease no elongated capillaries were found, 34 patients (79%) showing elongated capillaries. 16 patients - showed slight elongation, 1 moderate elongation and 10 marked elongation. All patients with early RA exhibited moderate or increased elongation. The other capilloscopic parameters were not influenced by disease duration.

The presence of erosions does not seem to influence the capilloscopic pattern. No correlation has been identified between the presence of erosions and capilloscopic changes.

Smoking does not appear to influence microvascular changes in patients with RA. The results do not confirm any significant association with the microvascular changes. The number of smoker patients included in the study was limited (12) thus, this finding should not be taken into account due to the fact that it might be false- negative.

Disability - quantified by the HAQ-DI - correlated with capillary distribution, elongation, tortuosity and subpapillary venous plexus visibility. No correlations were found between disability and capillary loop diameter (p Chi = 0.076, p Student = 0.100 > 0.05).

ESR and CRP correlated with elongation, tortuosity, subpapillary venous plexus visibility, capillary distribution and capillary loop diameter (dilated capillaries) and did not correlate with capillary density.

No correlation was found between DAS28 (4v)-CRP and capillary density (p Chi square = 0.362, p Student test = 0.109). We found high correlation between DAS28 (4v) -CRP and capillary distribution (p Chi = 0.000001, p Student <0.001). Significant correlations were also found between DAS28 (4v) -CRP and capillary length, (p Chi = 0.000007, p ANOVA <0.001), subpapillary venous plexus visibility (p Chi square = 0, 000057, p Student <0.001), tortuosity (p Chi, p Student <0.001) and dilated capillaries (p Student = 0.024)

DAS28 (4v)-CRP, CRP and ESR seem to influence the capillaroscopic parameters most. Thus, DAS28 (4v)-CRP significantly correlates with capillary distribution, elongation, tortuosity and visibility of subpapillary venous plexus. CRP and ESR are the only biological parameters that correlate with the identification of dilated capillaries. Also, CRP and ESR correlated significantly with capillary distribution, capillary elongation, tortuosity, and subpapillary venous plexus visibility.

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The results confirm the role of CRP in the process of endothelial activation and dysfunction, process possibly expressed as altered morphological and hemodynamic status of nailfold microvasculature.

The only capillary parameter uninfluenced by DAS28 (4v) -CRP, CRP and ESR was capillary density. In fact, it appears that normal or quasi-normal capillary density can distinguish between the RA and specific scleroderma capillaroscopic patterns.

RF influences on the capillaroscopic pattern were insignificant compared to the systemic inflammation and DAS28 (4v) -CRP. The RF titers correlated with capillary elongation (p Chi square = 0.40, p ANOVA = 0.007 <0.05), subpapillary venous plexus visibility (p Chi square = 0.009 <0.05, p ANOVA = 0.032) and tortuosity (p Chi square = 0.0083, p ANOVA = 0.004). The RF titer influences the capillary conformation, RF-positive patients, and predominantly those with high-titer RF, exhibiting a capillaroscopic pattern characterized by increased elongation and tortuosity. 89% of seropositive patients had elongated capillaries; those with RF > 10 X ULN (ULN = 14U / L) had moderate or marked elongation. Approximately 80% of RF positive patients had tortuosity; those with RF > 10X ULN showed moderate tortuosity at more than 3 fingers.. Also RF correlated subpapillar venous plexus subpapillar visibility. RF did not influenced capillary density, distribution and capillary loop diameter. ACPA antibodies did not influenced any of the capillaroscopic parameters.

When ACPA titers were compared we found that for capillary dilation and capillary density there were no significant differences (Student P test > 0.005), whereas for the capillary organization, elongation, tortuosity and visibility of the subpapillary venous plexus there were significant differences between each the four degrees of parameter (Student p or ANOVA <0.05).

This study sets opportunities for expanding research. The present research did not analyze the possible correlations between synovial inflammation and capillaroscopic parameters. Therefore it would be of interest to study the relation between the degree of synovial inflammation - using power Doppler joint ultrasonography and microvascular changes.

Also, given that RF and ACPA do not appear to have significant influence on the capillaroscopic pattern, it would probably be useful to test correlations with other immunomarkers - anti-CarP and anti-RA33 antibodies - and microvascular changes.

Also, if nailfold microvascular changes are considered as a sign of endothelial activation and dysfunction, it may be useful to test the relation between systemic inflammation (CRP), microvascular changes and serum markers of endothelial dysfunction - sICAM, ET-1, etc. Moreover, the previous hypothesis, corroborated with the evidence of increased cardiovascular risk and

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significant incidence of ischemic coronary disease in patients with RA, could justify studying the utility of capillaroscopy for assessing microvascular heart involvement in these patients.

Given that the results of this research and the vast majority of information available in literature conclude that in patients with RA, the capillaroscopic pattern is non-specific and distinct from that found in scleroderma patients, we might consider the feasibility of capillaroscopy in the assessment protocol of patients with undifferentiated early arthritis.