"OVIDIUS" UNIVERSITY CONSTANTA

FACULTY OF MEDICINE

# CONSIDERATIONS ON THE EARLY DIAGNOSIS OF RHEUMATOID ARTHRITIS

- ABSTRACT -

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# INTRODUCTION

Rheumatoid arthritis (RA) represents a pathology that affected man since antiquity, despite the fact that it has reached the incidence peak only in last centuries. Garrod was the first one that made the difference between RA and other rheumatological diseses, like gout and rheumatic fever. Today, both incidence and severity of the disease are declining.

Even though the causes of occurance are yet not known completely, there were identifyed several posible factors involved. Important etiological elements were introduced in selecting unique features in populations considered pre-disposed to RA. Along with environmental factors are contributing genetical, hormonal, infectious factors and a large set of other variables.

Due to the new diagnostic and therapeutically options, the number of patients in advanced, severe stages has been reduced lately. Maybe the most important element into fulfilling this goal is the establishment of a complex, complete and rapid diagnosis that allows an optimal therapy and an efficient management of evolution under treatment. Then, the practitioner's goal becomes the remission and even the cure of the disease.

The basic rationale of present research is motivated on one hand by the incidence of rheumatoid arthritis, still high in our country, and the opportunity of modern therapeutically options in early stages, and on the other hand, by the revolution of genetic research in the field made on solid background for this particular pathology. The new ACR/EULAR 2010 classification criteria were released in order to be able to minutiously analyze and classify early cases, especially for those with high risk of persistent and destructive forms that might beneficiate of disease modifying therapies.

Today, the diagnosis has the chance of going over the limit of conventional clinical and radiological criteria which are sufficiently positive only in late stages of disease, by this wasting time in implementation of new disease modifying and biological therapies. „Everything is new, but still old", said one of our great writers Eminescu, giving the definition of an absolute truth, applicable also in etiopathogenic research for rheumatoid arthritis, which, even if it was the objective of interest in hundreds of international studies and trials, still remains in the attention of medical world. Indeed, is needed a new, integrate vision that brings the results of fundamental research into the hands of practitioner, as an optimal tool for the diagnosis, treatment and prevention of the disease. Optimizing the management is no longer and ideal goal and the quality of life for these patients can be improved by establishing a clinical-genetical-biochemical-imagistical diagnosis that allows a better integration and a global reduction for medical costs.

Along with previous said, the lack of national trials and studies that might offer a genotipical pattern for rheumatoid arthritis patient and the present access to modern biological and imagistic investigational tools, are creating the rationales for initiating present study.

# PERSONAL CONTRIBUTIONS

The special part comprises two research lines, systematized under the form of two studies:

- Comparative analysis of classical ACR 1987 classification and of the new, modern ACR/EULAR 2010 classification criteria, in tight correlation with clinical, biological and imagistic markers, and
- The contribution of analysing the genetic factor in refining the diagnosis and evaluation of prognosis for patients with early rheumatoid arthritis, by integrative correlation with clinical, biological and imagistic markers.

**Objectives - study I**

Study I makes a detailed analysis of existing classification criteria used in research for rheumatoid arthritis, involving the classical and so much investigated set defined by ACR 1987 classification criteria, but more important the modern set ACR/EULAR 2010 that allows early inclusion of patients, before permanent structural articular damage, correlated with biological and imagistic markers.

The innovative feature is given by the comparative analysis of the criteria in the two sets that enlightens those most correlated with elements of severity, considering the important background of first classification and the need of adjustment for scientific results in the second one.

Main objectives are:

1. Describing population features for patient groups in terms related to age, sex, area of residence, ethnic, period of onset, date of establishing diagnosis based on corroborating clinical, biochemical and imagistic data; associated factors, heredocollateral history and associated pathologies analysis;
2. Analysing the disease activity indicators, defining principal articular areas involved, direct and comparative analysis of radiological and ultrasonografic articular changes;
3. Analysing the variable criteria used for the classification of patients with early rheumatoid arthritis, needed for diagnosis, corroborated with biochemical results, and secondary, establishing correlations between classical and new criteria.

**Objectives - study II**

The innovative element of our study is given by the lack of national studies addressing to genetic susceptibility given by the carriage of various HLA-DRB1 alleles in different ethnic groups in Romania, mainly caused by the high costs involved in genotyping. This comes along with a personal vision that tries to associate the phenotipical expression given by main characteristics of disease, defined by the classification criteria, with genotypic background.

Main objectives of study II are:

1. describing the genetical pattern expressed by HLA-DRB1 typing in patients with early rheumatoid arthritis compared with a control group selected from general population (matched accordingly with demographical features) and in correlation with

clinical, biochemical and imagistic data; corroborate analysis with associated risk factors and disease activity indicators;

2. Analyzing the association of genetic background with each of the criteria in classical and new, modern classification sets (ACR 1987 and ACR/EULAR 2010), and secondary establishing correlations between genetic factor and implementation of disease modifying therapies, focused on adverse reactions.

# MATERIAL AND METHOD

In order to satisfy research requests and objectives it was selected a group of 50 patients diagnosed with early rheumatoid arthritis according to ACR/EULAR 2010 classification criteria. Study included patients evaluated in Rheumatology Department of Central Clinical Emergency Hospital Constanta, respectively in out-patient facility of IOWEMED MEDICAL Center, Supplementary, for the second study it was necessary a population sample fromhealthy population, matched with age, sex and environment with first group.

Diagnosis was set based on ACR/EULAR 2010 classification criteria, considering joint involvement, serology, acute phase reactants and duration of symptoms. All patients presented a score $\geq 6$.

For the study group were retrospectively applied also ACR 1987 classification criteria.

Immunologic evaluation detected rheumatoid factor by latex-immunoturbidity with reference ranges of <14UI/mL, and anti-cyclic citrullinated peptides by FEIA with reference ranges of < 7 UI/mL – negative, 7-10 UI/mL – echivoc, > 10 UI/mL positive.

Imagistical evaluation included plain radiography, articular ultrasound and MRI.

Genomic DNA isolation and HLA-DRB1 genotyping were determined in Division of Genetics, Faculty of Medicine, "OVIDIUS" University, Constanta during 2012. Genotyping of HLA-DRB1 as low-resolution allele and HLA-DRB1*04 subgroups of allele as high resolution were determined by using PCR-SSP kits (InnoTrain Diagnostik GmbH, Germany; ProTrans produkte GmbH, Germany prin BioSupply) based on polymerase chain reaction, which enables amplification of DNA sequences, followed by sequence specific priming in which only the sequence of primer is responsible for the specification of the allele that has to be identified. Results of molecular detection was evaluated in agarose gel electrophoresis under UV light. Due to dimension of population groups, statistical analysis used chi-square test for evaluation of the statistical significance of differences in DRB1 allele frequencies in patients and in controls.

Statistical analysis assumed completing a database using SPSS v. 20.0 programme. Was considered statistically significant a $p$ value of $<0.05$.

# RESULTS

## Results - study I

Demographical features of patients with early rheumatoid arthritis enrolled in study are shown in table VIII.

Table VIII. Caracteristici demografice și biologice la pacienții cu AR precoce

| **Demographical and biological features** | No. = 50 |
|---|---|
| Age, mean +/- SD (years) | 58.54 ±12.223 |
| Onset <3 months (no., %) | 22 (44%) |
| Onset <12 months | 42 (84%) |
| Onset <36 months | 50 (100%) |
| Male (no., %) | 10 (20%) |
| Female (no., %) | 40 (80%) |
| Male:female ratio | 4:1 |
| Origin environment urban/rural | 37 (74%) / 13 (26%) |
| ACR/EULAR2010 score (media) | 7.24 ± 1.422 |
| RF positive | 34 (68%) |
| RF mean +/-SD (UI/mL) | 128.71 ± 158.474 |
| AntiCCP positive | 26 (52% of all) (57,77% of 45 with testing) |
| AntiCCP mean +/-SD (UI/mL) | 155.9 ± 175,044 |
| Smokers (nr., %) | 23 (46%) |
| Smokers/Non-smokers (ratio) | 23/27 (0,85) |
| Heterozygots / homozygots HLA-DRB1 | 45/5 |

All patients presented an onset shorter than 36 months up to the moment of diagnosis, and 84% were diagnosed in the first 12 months from onset, fulfilling the ACR/EULAR 2010 classification criteria. Less than a half (44%) presented an onset that allowed diagnosing in the first 3 moths of evolution. Male:female ratio was 4:1, with 80% in favour of female sex and a predominance of urban environmental origin compared with rural one (74%).

Smoking status presented a similar trend with a ratio of 0.85 in favour of non-smokers (only 46% patients were smokers). Smoking status correlated with environmental origin ($p = 0.030$), most smokers living in cities (Table IX).

Rheumatoid factor was found positive in 68% of patients with a calculated mean value of 128.71 UI/mL, and anti-cyclic citrullinated peptides antibodies in 56.80%, with a mean value of 155.9 UI/mL. So, sero-positivity in early RA patients reached 78%.

There was no statistically significant correlation between the presence of anti-cyclic citrullinated peptides and smoking ($p = 0.841$) nor between positivity for RF and smoking ($p = 0.113$), antiCCP antibodies being present in equal percentages in smokers and in non-smokers (table X). RF presented a slight increase in non-smokers (table XI).

Table X. Correlation between the presence of anti-CCP antibodies and smoking status

| | **Smokers** | **Non-smokers** |
|---|---|---|
| *AntiCCP present* | 12 | 13 |
| *AntiCCP absent* | 9 | 11 |
| | p= 0.841 | |

| | |
|---|---|
| Odds ratio | 1.1282 |
| 95 % CI | 0.3468 to 3.6705 |

- 5 patients did not perform antiCCP testing

Table XI. Correlation between the presence of rheumatoid factor and smoking status

| | **Smokers** | **Non-smokers** |
|---|---|---|
| *RF present* | 13 | 21 |
| *RF absent* | 10 | 6 |
| | p= 0.113 | |

| | |
|---|---|
| Odds ratio | 0.3714 |
| 95 % CI | 0.1090 to 1.2656 |

The most important correlation in demographical features analysis was the one showing the status of seropositivity / seronegativity for antiCCP and RF. AntiCCP antibodies correlated significantly with rheumatoid factor – p = 0.008 (table XII). Only 11 patients were negative for both antibodies.

Table XII. Correlation between presence of rheumatoid factor and anti-cyclic citrullinated peptides antibodies

| | **AntiCCP +** | **AntiCCP -** |
|---|---|---|
| *RF present* | 21 | 9 |
| *RF absent* | 4 | 11 |
| | p= 0.008 | |

*(5 patients did not perform anti CCP testing)

| | |
|---|---|
| Odds ratio | 6.4167 |
| 95 % CI | 1.6056 to 25.6445 |

Environmental origin (urban/rural) was not significantly associated neither with the presence of rheumatoid factor (p = 0.423), nor with the presence of anti-cyclic citrullinated peptide (p = 0.938) (Table XIII).

Table XIII. Correlation between environmental origin and presence of anti-CCP antibodies

| | **AntiCCP +** | **AntiCCP -** |
|---|---|---|
| *Urban* | 19 | 15 |
| *Rural* | 6 | 5 |
| | p= 0.938 | |

| | |
|---|---|
| Odds ratio | 1.0556 |
| 95 % CI | 0.2692 to 4.1388 |

Our study shown a neat difference in higher activity scores in males with onset shorter than 3 months compared with the ones presented later.

Table XIV. Demographical and biological features in patients with early rheumatoid arthritis divided by sex

| **Demographical and biological features** | **Male** No. = 10 (20%) | **Female** No. = 40 (80%) |
|---|---|---|
| Age, mean +/- SD (yrs) | 62.40 ±12.859 | 57,25±11.833 |
| Urban/rural | 7/3 | 30/10 |
| ACR/EULAR2010 score (mean) | 7.50 ± 1.354 | 7.27 ± 1.501 |
| RF positive | 6 (60%) | 29 (72,50% of no.) |
| RF mean +/-SD (UI/mL) | 128.60 ± 231,168 | 128,69±138.681 |
| AntiCCP positive | 5 (50% of no.) (11,11% of pac.performing test) | 21 (58,33% of no.) (46,66% of pac.performing test) |
| AntiCCP mean +/-SD (UI/mL) | 157.65 ± 181.529 | 141,07±176.030 |
| Smokers (no., %) | 8 (80%) | 15 (37,50%) |
| Smokers/non-smokers (ratio) | 8/2 (4) | 15/25 (0,6) |
| ESR mean +/-SD (mm/h) | 49,50±24.99 | 46,62±25.045 |
| CRP mean +/-SD (UI/mL) | 5,88±3.255 | 2,84±3.438 |
| DAS 28– mean (min-max) | 6.646±0,892 | 6,170±0.9916 |

Mean value for RF was practically similar in males and females, comparative with mean value observed for antiCCP antibodies that was higher in males (table XIV). Interesting were the values of acute phase reactants superior in range in case of male patients.

Table XV. Correlation between sex and serology for antiCCP antibodies

| | **Female** | **Male** |
|---|---|---|
| *antiCCP present* | 21 | 5 |
| *antiCCP absent* | 19 | 4 |
| | p= 0,868 | |

| | |
|---|---|
| Odds ratio | 0.8842 |
| 95 % CI | 0.2066 to 3.7842 |

- 4 females did not perform antiCCP testing; 1 male did not perform antiCCP testing

Table XVI. Correlation between sex and serology for RF

| | **Female** | **Male** |
|---|---|---|
| *RF present* | 29 | 7 |
| *RF absent* | 11 | 3 |
| | p= 0.874 | |

| | |
|---|---|
| Odds ratio | 1,129 |
| 95 % CI | 0.2471 to 5.1671 |

Correlations between the presence of different antibodies and the sex of the patients with early rheumatoid arthritis (table XV and XVI), proved to be not statistically significant, even if there were noted differences between sub-groups separated by sex (table XIV) related to sero-positivity to antibodies.

In this way, 40% of male patients were positive for RF, compared with only 27,50% of female patients. In case of antiCCP antibodies, if in male patients the proportion was equal, in female patients the percentage was little lower of 41,66%.

80% of male patients were smokers and only 37,50% of female were smokers.

Smoker status did not correlate significantly with RF or anti-CCP in sub-groups separated by sex, non-smoker females being more positive for RF (p = 0,523; OR = 0.63; 95%CI = 0.15-2,59). In case of males, smokers were more sero-negative for RF, but also in this case statistically insufficient. (p = 0,223; OR = 0.12; 95%CI =0.004 to 3,51). Same trend was observed antiCCP antibodies (figure 25).

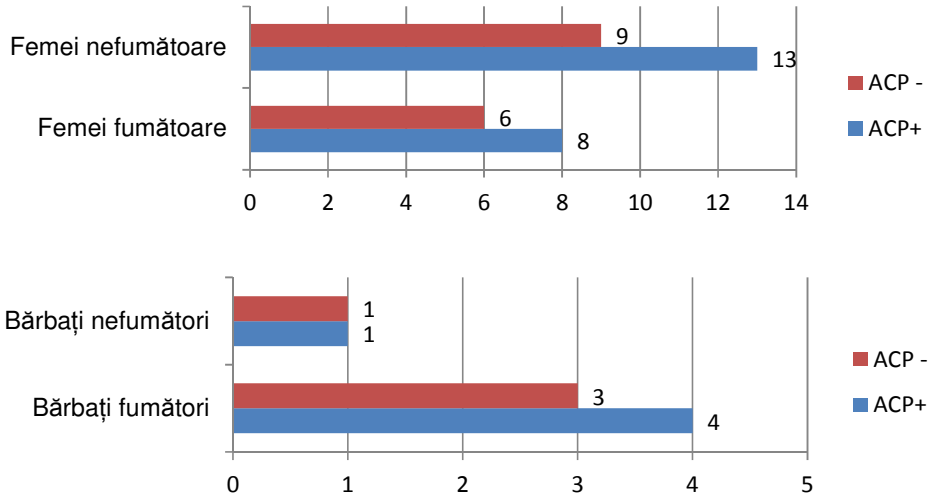



*Figure 25. Correlations between smoking and positive serology for ACP divided into sex groups (ACP – antiCCP antibodies)*

Generally, the serology did not correlate significantly with the sex of the patients (for FR – p = 0,874, respective for antiCCP – p = 0,868).

More than a half of male patients were positive for RF or anti-CCP antibodies, and 40 % were negative compared with 22% percentage in general group, showing an independent status compared with females. 80% of male patients with early rheumatoid arthritis were smokers, half of them being positive for anti-CCP antibodies, and 70% positive for RF. The titre of antibodies is interesting, following the trend in the titres demonstrated in general analysis, exactly 128,6 UI/mL for RF (compared with 128,71 UI/mL in general group) and of 157,65 UI/mL for anti-CCP (compared with 155,9 UI/mL in general group).

Antibodies were slightly higher in females compared with males – 72,50% for FR, and antiCCP in a percentage of 58,33%.

80 % of the patients mentioned no heredocollateral history of rheumatismal diseases or collagenoses, compared with the rest of 14% that presented cases of rheumatoid arthritis in close relatives (mother, father, sister/brother) and only 6% with other rheumatismal pathologies (especially psoriasis).

Associated diseases in studied patients, reveals a high incidence for cardiovascular and endocrine pathologies and in smaller amount genitor-urinary ones.

Biological syndrome of inflammation showed a mean for erythrocyte sedimentation rate of 46.28 mm/h +/- 25.059 and of 3.28 UI/mL +/-3.454 for C reactive protein. Along with this, in table XVII are summarized values of VAS (visual analogue scale) with a mean score of 67,9, but also for DAS 28 with a mean score of 6.25 and with a standard deviation close to 1 (0.957), demonstrating a 88% of patients with highly active form of disease, compared with moderate and low active forms.

Table XVII. Disease activity parameters in early RA patients

| **Parameters** | | No. = 50 |
|---|---|---|
| No . tender joints – mean (min-max) | | 17.96 ± 11.514 (min 3- max 47) |
| No. swollen joints – mean (min-max) | | 7.32 ± 6.384 (min 1- max 34) |
| VAS – mean (min-max) | | 67.90 ± 16.228 (min 40- max 100) |
| DAS 28– mean (min-max) | | 6.251 ± 0.957 (min 4.22- max 8.21) |
| High active: >5.1 (nr.-%) | | 44 (88%) |
| Moderate active: 3.2-5.1 (no. - %) | | 6 (12%) |
| Extra-articular manif. - total (no. - %) | | 15 (30%) |
| Type of extra-articular manif.(no. – % of total no.) | Chronic anaemia | 9 (18%) (45% of ex.ar.manif.) |
| | Pulmonary changes | 4 (8%) (20% of ex.ar.manif.) |
| | Carpal tunnel sdr. | 4 (8%) (20% of ex.ar.manif.) |
| | other | 3 (6%) (15% of ex.ar.manif.) |
| ESR mean +/-SD (mm/h) | | 46.28 ± 25.059 |
| CRP mean +/-SD (UI/mL) | | 3.28 ± 3.454 |

Extra-articular manifestations joined 30% of the cases with early RA, in which the highest proportion was represented by chronic anaemia, in contrast with other like carpal tunnel syndrome and pulmonary manifestations. (table XVII).

In this particular sub-group with extra-articular manifestations, rheumatoid factor was positive in 66,66% case (almost similar with general group), but with a mean calculated range of 169,28 UI/mL. More than that, apparently convergent, the percentage of sero-positivity for anti-cyclic citrullinated antibodies was 53,33%, with a neat superior mean average of 225 UI/mL.

Mean number of tender joints was 17.96, with minimum of 3 and a maximum of 47 involved joints, and the number of swollen joints summed an average of 7.32 +/- 6.384.

The distribution of articular areas revealed as principal metacarpophalangian group, proximal interphalangian joints, radiocubitocarpal joints, but also the knee joint area, compared with main swollen joint areas represented by radiocubitocarpal joint and metacarpophalangian joints.

Imagistic investigation of patients with early RA allowed revealing the structural changes in articulation by the use of plain radiograph and ultrasonographic exam. Main radiographic changes are presented in table XVIII, showing in 20% juxta articular osteoporosis, and resembling 28.57% of the 35 patients undergoing investigation. In lesser percentages of 22.85% and respective 8.57% of radiologically assessed patients were found erosive changes and joint deformities. Important to notice is the favourable ratio for seropositive patients with radiographic changes (16/5 – 3.2 sero+/sero- patients), even if each type of positive serology calculated separately did not correlate statistically significant with radiographic changes ($p = 0.719$ for antiCCP and $p = 0.999$ for RF).

Table XVIII. Radiographic changes in early RA patients

| **Radiographic changes** | **Nr. = 35 pacienți care au efectuat Rx** |
|---|---|
| Patients with radiographic changes/patients without radiographic changes | 21:14 (1.5) |
| Erosions/geodes (no., %) | 8 (16% of 50, 22.85% of 35 with Rx) |
| Juxta articular osteoporosis (no., %) | 10 (20% of 50, 28.57% of 35 with Rx) |
| Displacements/joint deforming (no., %) | 3 (6% of 50, 8.57% of 35 with Rx) |
| Sero+/sero- ratio with radiographic changes | 16/5 (3.2) |

It was observed a high frequency of non-smokers with radiographic changes, even if the differences were not statistically significant compared with smokers (p = 0.487) (table XIX).

Tabelul XIX. Correlation between smoking and radiographic changes

| | **Smokers with Rx** | **Non-smokers with Rx** |
|---|---|---|
| *Rx changes* | 6 | 11 |
| *No Rx changes* | 8 | 9 |
| | p= 0.487 | |

| | |
|---|---|
| Odds ratio | 0.6136 |
| 95 % CI | 0.1548 to 2.4322 |

In case of changes seen by ultrasound investigation, the distribution was different (table XX). The sero+/sero- ratio in patients with ultrasonographic changes regardless joint area was only 1.22 (11/9) and maintained the same trend of 1-1.28 at separate analysis of distinct joint area. 82.6% of patients that undergo ultrasonography for different joint areas presented synovial/proliferations (93.8% of those that undergo hand joint US and 57.14% of those for other areas), 69.56% presented tendinitis/tenosynovitis/tendon rupture (87.5% of those that undergo hand joint US and 28.57% of those for other areas), and only 4.34% presented recallable bone erosions (6.3% of those with hand joint US).

Table XX. Ultrasonographic changes in early RA patients

| **Ultrasonographic changes in hand joints** | **No. = 16 patients with hand joint US** |
|---|---|
| Synovial effusion/proliferation (no., %) | 15 (30% of 50, 93.8% of 16 with US) |
| Tendinitis/tenosynovitis/tendon rupture (no.,%) | 14 (28% of 50, 87.5% of 16 with US) |
| Erosions (no., %) | 1 (2% of 50, 6.3% of 16 with US) |
| Sero+/sero- ratio with ultrasonographic changes | 9/7 (1.28) |
| **Ultrasonographic changes in other joint areas** | **No. = 7 patients with other joint areas US** |
| Synovial effusion/proliferation (no., %) | 4 (8% of 50, 57.14% of 7 with US) |
| Tendinitis/tenosynovitis/tendon rupture (no.,%) | 2 (4% of 50, 28.57% of 7 with US) |
| Erosions (no., %) | 0 |
| Sero+/sero- ratio with ultrasonographic changes | 2/2 (1) |
| **Ultrasonographic changes regardless joint type (total)** | **No. = 23 patients undergoing US** |
| Patients with ultrasonographic changes/patients without ultrasonographic changes | 20:3 (6.66) |
| Synovial effusion/proliferation (no., %) | 19 (38% of 50, 82.6% of 23 with US) |
| Tendinitis/tenosynovitis/tendon rupture (no.,%) | 16 (32% of 50, 69.56% of 23 with US) |
| Erosions (no., %) | 1 (2% of 50, 4.34% of 23 with US) |
| Sero+/sero- ratio with ultrasonographic changes | 11/9 (1.22) |

AntiCCP corr.with ultrasound changes: p=0.096; RF corr.with ultrasound changes: p=0.230

Anti-cyclic citrullinated peptides correlated better with ultrasonographic changes than rheumatoid factor, yet insufficient to be statistically significant (p = 0.096 for antiCCP, compared with 0.230 for RF).

Total ratio of patients with ultrasonographic changes / without ultrasonographic changes was clearly superior than the one present for radiographic changes (6.66 vs. 1.5).

Comparative analysis of variable criteria used for the classification of rheumatoid arthritis is presented in table XXI. ACR 1987 classification criteria were fulfilled only by 60% (even if applied retrospectively), compared with ACR/EULAR 2010 fulfilment of 100%. We have to mention that this is not surprising facing the fact that the inclusion criteria were extremely strict regarding differential diagnosis.

Interesting was to calculate the frequency of each criterion that revealed equal proportions (≈90%) for criteria 1 and 3 (arthritis of 3 or more joints and arthritis of hand joints) in ACR 1987 set and criterion C (acute phase reactants) in ACR/EULAR 2010 classification.

Related to ACR 1987 criteria in early RA patients, field was dominated by arthritis of hand joints, symmetry, morning stiffness and RF positivity. The poorest

presentation was for radiographic changes and rheumatoid nodules (18% and respective, 2%).

ACR/EULAR 2010 criteria analysis showed a mean total score of 7.34 (practically synonymous with a positive diagnosis for all patients); 40% of patients presented at least 10 joints involved, 56% high titres for RF or antiCCP, 92% a biological syndrome of inflammation characterized by high ESR or CRP and 84% a persistence of symptoms over 6 weeks (table XXI).

Table XXI. ACR 1987 vs. ACR/Eular 2010 criteria analysis

| **ACR 1987 classification criteria** | | **ACR/EULAR 2010 classification criteria** | |
|---|---|---|---|
| Fulfilled criteria - total (no.%) | 30 (60%) | Fulfilled criteria - total (no.%) | 50 (100%) |
| | | Score (mean +/-SD) | **7.34 +/- 1.422** |
| **1. Arthritis of 3 or more joint areas** | 45 (90%) | **A. Joint involvement** (mean score +/- SD) | **3.5 +/- 1.359** |
| | | 1 large joint | 0 (0%) |
| **2. Morning stiffness ≥ 60 min** | 31 (62%) | 2-10 large joints | 4 (8%) |
| | | 1-3 small joints (+/- large joints) | 7 (14%) |
| **3. Arthritis in hand joints** | 45 (90%) | 4-10 small joints (+/- large joints) | 19 (38%) |
| | | >10 joints (at least 1 small joint) | 20 (40%) |
| **4. Symmetric arthritis** | 36 (72%) | **B. Serology** (mean score+/- SD) | **2.08 +/- 1.309** |
| | | RF neg. and antiCCP neg. | 11*(24%) |
| **5. Rheumatoid nodules** | 1 (2%) | RF or antiCCP pos. low titer | 10 (20%) |
| | | RF or antiCCP pos.high titer | 28 (56%) |
| **6. Positive RF** | 34 (68%) | **C. Acute phase reactants** (mean score+/- SD) | **0.92 +/- 0.274** |
| | | ESR and CRP normal | 4 (8%) |
| | | ESR and CRP high | 46 (92%) |
| **7. Radiographic changes** | 9 (18%) | **D.Duration of symptoms** (mean score+/- SD) | **0.84 +/- 0.370** |
| | | < 6 weeks | 8 (16%) |
| | | ≥ 6 weeks | 42 (84%) |

* 1 RF seronegative patient did not undergo antiCCP testing.

ACR/EULAR 2010 criteria presented a better correlation with smoker/non-smoker status than ACR 1987 criteria, but these correlations were not statistically significant (ACR 1987 per total with smoking status: p= 0.105; ACR/EULAR 2010 per total with smoking status: p= 0.099).

Analysing biological syndrome of inflammation enlightened by acute phase reactants ESR and CRP from ACR/EULAR 2010 criteria, this was statistically significant correlated with disease activity score DAS28 (p = 0.045) and more important with extra-articular manifestations in patients with early rheumatoid arthritis (p=0.0507). Correlations with RF and antiCCP antibodies were not statistically significant (p = 0.578 and respective p = 0.842), nor the correlations with radiographic changes (p = 0.999). Symmetric arthritis in classic ACR1987 criteria presented the best correlation with biological syndrome of inflammation (p = 0.021), implicitly with ESR and CRP (p = 0.026, respective p = 0.017) and extremely significant (p = 0.002) with disease activity score DAS28 (table XXII).

The poor correlation with ultrasonograohic changes of this criterion (p = 0.082), was sufficient to note that 78.26% of patients with symmetrical arthritis presented ultrasonographic changes. Morning stiffness and arthritis of hand joints criteria correlated with high values of CRP (p = 0.039, respective 0.018) (table XXII). Morning stiffness criterion did not correlate nor as presence or as value with none of the new ACR/EULAR 2010 criteria (table XXIII).

Table XXII. Correlation between ACR 1987 classification criteria with disease activity indicators, acute pahse reactants and ultrasonographic changes

| ACR 1987 criteria | ESR | CRP | DAS28 | Ultarsonographic changes |
|---|---|---|---|---|
| **Arthritis of 3 or more joint areas** | p=0.102 | p=0.447 | p=0.045 | p=0.923 |
| **Morning stiffness** | p=0.645 | p=0.039 | p=0.689 | p=0.183 |
| **Arthritis of hand joints** | p=0.120 | p=0.018 | p=0.073 | p=0.923 |
| **Symmetric arthritis** | p=0.026 | p=0.017 | p=0.002 | p=0.082 |
| **Rheumatoid nodules** | p=0.917 | p=0.200 | p=0.808 | p=0.429 |

Table XXIII. Correlation between ACR/EULAR 2010 classification criteria with morning stiffness, symmetric arthritis and radiographic changes

| ACR/EULAR 2010 criteria | Morning stiffness | Symmetric arthritis | Radiographic changes |
|---|---|---|---|
| **Joint involvement** | p=0.890 | p=0.009 | p=0.174 |
| **Serology** | p=0.531 | p=0.454 | p=0.048 |
| **Acute phase reactants (ESR;CRP)** | p=0.106 | p=0.021 | p=0.086 |
| **Duration of symptoms** | p=0.413 | p=0.134 | p=0.152 |

## Results - study II

An extremely relevant analysis for the diagnostic and prognostic implications is represented by the study of genetic features of patients with early rheumatoid arthritis, a case-control study made bias a significant control group from general healthy population.

Tables XXIV and XXV present the distribution of HLA-DRB1 alleles observed in patients compared with controls. HLA-DRB1 genotyping showed statistically significant differences both for potentially containing shared epitope alleles and for other alleles. The most important were represented by the statistically significant high frequency of *04 (17% vs. 8.98%; p = 0.0474) and *10 (5% vs. 1.12%; p = 0.0477) alleles, but also for the rest of potentially containing SE like *01 (15% vs. 8.42%; p = 0.0900) allele. *14 presented a comparable frequency with control group, with a non-significant difference. The general distribution of potentially containing SE alleles was striking, our study determining a high frequency in early RA group (44% vs. 21.91%; p = <0.0001). High frequency, but not sufficiently statistic presented also a suspect allele - *07 (7% vs. 3.37%; p = 0.1689).

Reduced frequencies compared with controls were found for *11 (12% vs. 20.22%, p = 0.0816), *13 (6% vs.14.60%; p < 0.0001) and *15 alleles (5% vs. 12.35%, p = 0,0467), all statistically significant.

With minor percentage differences, the total frequency of homozygots in RA group did not differ statistically significant compared with control group (p = 0.8480), with similar findings in case of homozygots potentially containing SE (*01, *04, *10, *14; p = 0.8497) or the carriage of two different alleles potentially containing SE (table XXIV).

Table XXIV. The frequencies of HLA-DRB1 alleles in patients with early rheumatoid arthritis (ERA) and controls

| HLA-DRB1 alleles | ERA (2 x no. = 100) | | Controls (2 x no. = 178) | | p (<0.05) | O.R | 95%CI |
|---|---|---|---|---|---|---|---|
| | No. | Freq. (%) | No. | Freq. (%) | | | |
| *01 | 15 | 15% | 15 | 8.42% | 0.0900 | 1.91 | 0.89-4.10 |
| *03 | 14 | 14% | 24 | 13.48% | 0.9041 | 1.04 | 0.51-2.12 |
| *04 | 17 | 17% | 16 | 8.98% | 0.0474 | 2.07 | 0.99-4.31 |
| *07 | 7 | 7% | 6 | 3.37% | 0.1689 | 2.15 | 0.74-6.60 |
| *08 | 0 | 0% | 2 | 1.12% | - | | |
| *10 | 5 | 5% | 2 | 1.12% | 0.0477 | 4.63 | 0.88-24.32 |
| *11 | 12 | 12% | 36 | 20.22% | 0.0816 | 0.53 | 0.26-1.08 |
| *12 | 1 | 1% | 0 | 0.00% | - | | |
| *13 | 6 | 6% | 26 | 14.60% | 0.0309 | 0.37 | 0.14-0.94 |
| *14 | 6 | 6% | 6 | 3.37% | 0.3005 | 1.82 | 0.57-5.83 |
| *15 | 5 | 5% | 22 | 12.35% | 0.0467 | 0.37 | 0.13-1.01 |
| *16 | 12 | 12% | 23 | 12.92% | 0.8241 | 0.91 | 0.43-1.93 |
| Carriage of 2 SE allele | 9 | 18% (of no.) | 10 | 11,23% (of no.) | 0.2835 | 1.66 | 0.65-4.23 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Homozygots global | 5 | 10% (of no.) | 8 | 8,98% (of no.) | 0.8480 | 1.11 | 0.35-3.51 |
| Homozygots potentially SE | 2 | 4% (of no.) | 3 | 3,73% (of no.) | 0.8497 | 1.19 | 0.19-7.24 |
| Main potentially containing SE alleles (sum *01,*04,*10,*14) | 44 | 44% | 39 | 21.91% | <0.0001 | 2.80 | 1.64-4.76 |

Related to the distribution of HLA-DRB1*04 sub=group alleles, estimation was little difficult due to small of sample that implied the use of binomial exact test. This revealed a statistically significant frequency for *04:04 (23.52% vs. 6.25%; p = 0.0003), but also for *04:08 alleles, even if in this late case without statistical relevance (11.76% vs. 6.25%; p = 0.1382) – significance is given also by the superior O.R. value for *04:04 and *04:08 (4,61, respective 2). In contrast, *04:02 were found significantly low in patients with early RA (11.76% vs. 31.25%; p < 0.00001). *04:01, *04:03 and *04:05 sub-group alleles were slightly reduced as frequency, but the difference is not statistically significant (table XXV).

Tabelul XXV. The frequencies of HLA-DRB1*04 subgroup alleles in patients with early rheumatoid arthritis and controls

| DRB1*04 sub-group alleles | ERA (no*04 = 17) | | Controls (no *04 = 16) | | p (<0.05) | O.R | 95%CI |
|---|---|---|---|---|---|---|---|
| | No. | Freq. (%) | No. | Freq. (%) | | | |
| *04:01 | 3 | 17.64% | 3 | 18.75% | 0.8555 | 0.92 | 0.15-5.44 |
| *04:02 | 2 | 11.76% | 5 | 31.25% | <0.00001 | 0.29 | 0.04-1.80 |
| *04:03 | 2 | 11.76% | 2 | 12.5% | 0.8306 | 0.93 | 0.11-7.55 |
| *04:04 | 4 | 23.52% | 1 | 6.25% | 0.0003 | 4.61 | 0.45-46.67 |
| *04:05 | 3 | 17.64% | 3 | 18.75% | 0.8555 | 0.92 | 0.15-5.44 |
| *04:06 | 0 | 0% | 1 | 6.25% | | | |
| *04:07 | 1 | 5.88% | 0 | 0% | | | |
| *04:08 | 2 | 11.76% | 1 | 6.25% | 0.1382 | 2.00 | 0.16-24.48 |

Calculated relative risk (figure 31) for studied group allowed a stratification of main potentially containing shared epitope alleles. In patients with early RA, the highest relative risk was found in *10 allele (RR = 4.63), followed by *04 (RR = 2.07), and on the last places being *01 and *14 with RR = 1.91, respective RR = 1.81. It was calculated also the relative risk for (figure 32) HLA-DRB1*04 sub-groups, in which the highest value was found for *04:04 (RR = 4.34), compared with lower risks in *04:08 (RR = 2) or *04:01 and *04:05 alleles (RR similar de 0.93).

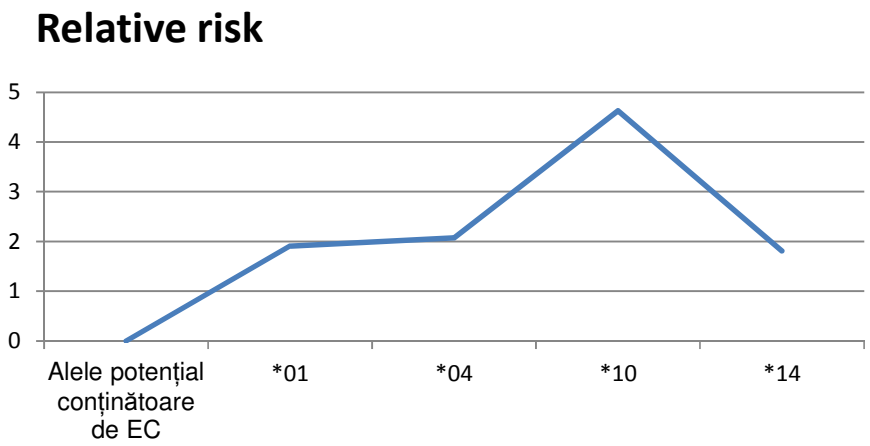



*Figure. 31. Relative risc for main potentially containing shared epitope alleles*

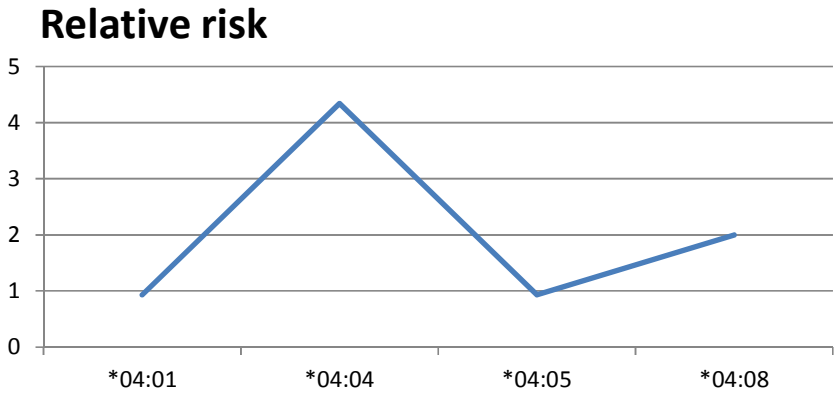



*Figure 32. Relative risk for HLA-DRB1*04 sub-group alleles*

The stratification of odds ratio brought into attention *10 allele (OR=4,63), followed by *04 and *07 alleles (OR=2,07 and respective OR=2,15) which presented a similar trend with potentially containing shared epitope alleles (OR=2,80). *01 and *14 were parallel similar in trend – OR=1,91 and respective OR=1,82.

Table XXVI. The frequencies of HLA-DRB1 alleles in smoker patients with early rheumatoid arthritis (ERA)

| HLA-DRB1 alleles | ERA (2 x no. = 46) | |
|---|---|---|
| | No. | Freq. (%) |
| *01 | 6 | 13,04% |
| *03 | 3 | 6,52% |
| *04 | 11 | 23,91% |
| *07 | 4 | 8,69% |
| *08 | 0 | 0% |
| *10 | 1 | 2,17% |
| *11 | 4 | 8,69% |
| *12 | 1 | 2,17% |
| *13 | 3 | 6,52% |
| *14 | 3 | 6,52% |
| *15 | 2 | 4,34% |
| *16 | 8 | 17,39% |
| Carriage of 2 SE allele | 4 | 17,3% (of no.) |
| Homozygots global | 3 | 13% (of no.) |
| Homozygots potentially SE | 2 | 8,69% (of no.) |
| Main potentially containing SE alleles (sum *01,*04,*10,*14) | 19 | 41,30% |

The most frequent alleles found in smoker patients with early RA were *04 (23,91%), closely followed by *01 allele (13,04%), and maybe the most intriguing aspect was given by the general frequency of main potentially containing SE alleles of 41,30%, pretty similar with the one found in general group analysis (Table XXVI). The frequency of *04 sub-group in smoker patients with dominant *04:04, *04:05 and *04:02 seem to complete the pattern of genetic risk factor, somehow parallel with the one in general analysis (Table XXVII).

Table XXVII. The frequencies of HLA-DRB1 sub-group alleles in smoker patients with early rheumatoid arthritis (ERA)

| DRB1*04 sub-group alleles | ERA (no. alleles *04 = 11) | |
|---|---|---|
| | No. | Freq. (%) |
| *04:01 | 1 | 9,09% |
| *04:02 | 2 | 18,18% |
| *04:03 | 1 | 9,09% |
| *04:04 | 3 | 27,27% |
| *04:05 | 2 | 18,18% |
| *04:06 | 0 | 0% |
| *04:07 | 1 | 9,09% |
| *04:08 | 1 | 9,09% |

From potentially containing shared epitope alleles, *04, *10 and *14 (table XXVIII) correlated statistically significant with the presence of anti-cyclic citrullinated peptides (p = 0.030, p = 0.038, respective p = 0.006), in contrast with *01 allele. *14 allele correlated sufficiently with the presence of rheumatoid factor (p = 0.05).

Proper analysis involved *13 allele considered to be protective for RA, but also *16 allele with a high presence in studied population (table XXVIII). Statistically significant was the negative association between the presence of *13 allele and rheumatoid factor (p = 0.052).

Table XXVIII. Correlations between HLA-DRB1 alleles with biologic parameters and disease severity indicators

| | **EC*01** | **EC*04** | **EC*10** | **EC*14** | ***16** | ***13** |
|---|---|---|---|---|---|---|
| **Radiographic changes** | p=0.999 | p=0.697 | p=0.999 | p=0.545 | p=0.999 | p=0.146 |
| **antiCCP** | p=0.797 | p=0.030 | p=0.038 | p=0.006 | p=0.985 | p=0.420 |
| **RF** | p=0.726 | p=0.318 | p=0.544 | p=0.050 | p=0.643 | p=0.052 |
| **High activity (DAS28 >5.1)** | p=0.756 | p=0.510 | p=0.384 | p=0.708 | p=0.217 | p=0.708 |
| **Extra-articular manifestations** | p=0.123 | p=0.304 | p=0.999 | p=0.214 | p=0.875 | p=0.722 |

Serologically negative patients had an interesting genetic distribution, the analysis showing a high frequency for *01 allele, compared with *04 or *14.

10% of studied patients with early rheumatoid arthritis were Asiatic ethnics, and was interesting to follow the phenotipical features of this particular sub-group, correlated with HLA-DRB1 genotype, even if the reduced sample size did not allow an important statistical analysis.

Dominant in Asiatic group was *04 allele, with equal distribution for *04:01, *04:04, *04:05, closely followed by *07 and *15, and lesser *01, *03 and *14.

The pattern of this group was interesting: only one Asiatic patient was negative for RF and/or ACP, 80% were seropositives for RF (with mean titre of 63,80 UI/mL ± 49.77), and only 60% were seropositives for antiCCP antibodies (with mean titre of 296,79 UI/mL ± 202.79).

We have to enlighten that the Asiatic patients presented lower RF titters compared with general group, and spectacularly high titters for antiCCP antibodies. Considering the high frequency for *04 allele (30%) compared with Caucasoid patients (15,55%), we performed the comparison between groups, but it did not reveal significant differences (table XXIX).

Table XXIX. The frequencies of HLA-DRB1 alleles in asiatic patients with early rheumatoid arthritis (ERA) vs. Caucasoid patients

| HLA-DRB1 alleles | Asiatic ERA aleles (2 x no. = 10) | | Caucasoid ERA alleles (2 x no. = 90) | | p (<0.05) | O.R | 95%CI |
|---|---|---|---|---|---|---|---|
| | No. | Freq. (%) | No. | Freq. (%) | | | |
| *04 | 3 | 30% | 14 | 15,55% | 0,2595 | 2,32 | 0,53-10,09 |
| Main potentially containing SE alleles (sum *01,*04,*10,*14) | 5 | 50% | 34 | 37,77% | 0,4556 | 1,64 | 0,44-6,10 |

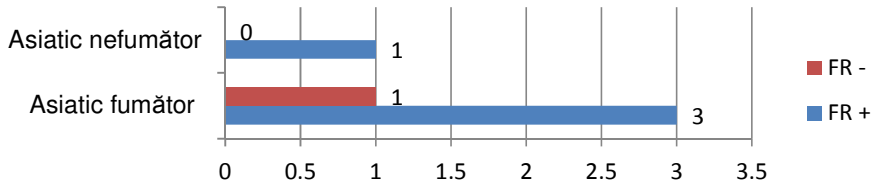
The ACR/EULAR 2010 classification criteria score was an average of 8 ± 1.22, and the disease severity indicators presented high ranges (table XXX).

If the correlation is made with entire patient group, the frequency of potentially containing SE alleles becomes statistically significant for Asiatic origin (p = 0,05; OR = 3,56 95% CI 0,98-12,94), with a higher risk ratio than in general patient group (3,56 vs. 2,80).

Table XXX. Severity indicators in asiatic patients with early RA

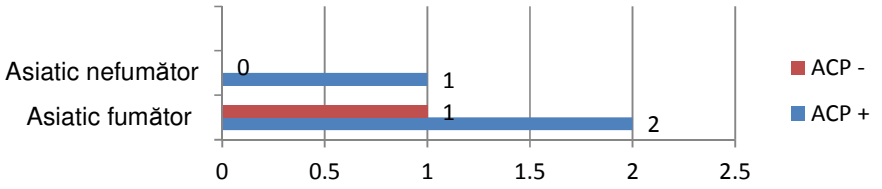
| **Indicators** | No. = 5 |
|---|---|
| DAS 28 – mean (min-max) | 6.72 ± 0.91 |
| ESR mean +/-SD (mm/h) | 57 ± 20.95 |
| CRP mean +/-SD (UI/mL) | 4.37 ± 3.52 |

From this group, 80% were smokers, with a female:male ratio of 3:2, all of them coming from urban environment.




| | |
|---|---|
| Odds ratio | 0.5556 |
| 95 % CI | 0.0126 to 24.5152 |
| | P = 0.7610 |

*Figure 37. Correlation between smoker status and positive serology for RF antibodies in Asiatic patients with early RA*




| | |
|---|---|
| Odds ratio | 0.7778 |
| 95 % CI | 0.0187 to 32.3738 |
| | P = 0.8949 |

*Figure 38. Correlation between smoker status and positive serology for anti-CCP antibodies in Asiatic patients with early RA (ACP- anti-CCP)*

Positive serology for RF or antiCCP antibodies did not correlate significantly with smoker status (figures 37 and 38).

40% of Asiatic patients presented ultrasonographic changes and 40% radiographic changes, all of them associating smoking. The correlation of imagistic changes with smoking in asiatic RA group was not statistically significant (p = 0,55), but the high risk ratio (OR = 3) seems to sustain the high risk of smoking Asiatic patients to develop severe, destructive forms. We have to add that only one patient of Asiatic origin presented extra-articular manifestations with pulmonary changes (pulmonary rheumatoid nodules associated with pleurisy), patient being non-smoker.

As an observation in asiatic patients, *04:05 sub-group presented radiographic changes, in contrast with *04:01 and *04:04 which presented ultrasonographic changes.

The analysis of homozygote status for patients presenting *01 or *04 potentially containing shared epitope alleles did not reveal statistically significant associations with serology (RF or antiCCP), radiographic changes or disease severity (Table XXXI).

Table XXXI. Correlations between HLA-DRB1 alleles and biological parameters and severity indicators in homozygots

| | **Homozygots SE*01** | **Homozygots SE*04** | **Homozygots *16** | **Homozygots *13** |
|---|---|---|---|---|
| **Radiographic changes** | p=0.310 | p=0.310 | - | - |
| **antiCCP** | p=0.246 | p=0.246 | - | - |
| **RF** | p=0.488 | p=0.488 | p=0.578 | - |
| **High activity score** | p=0.709 | p=0.709 | p=0.594 | - |
| **Extra-articular manifestations** | p=0.409 | p=0.409 | p=0.239 | - |

One of the most interesting analysis was represented by the observation of the correlations between potentially containing SE alleles, also *04 sub-group alleles and each criterion in ACR 1987 (tables XXXII and XXXIII) and ACR/EULAR 2010 classifications. (tables XXXIV and XXXV).

Table XXXII. Associations between ACR 1987 classification criteria and potentially containing SE alleles

| ACR 1987 criteria | Potentially containing SE alleles | *04 | *01 | *10 | *14 |
|---|---|---|---|---|---|
| **Arthritis ≥ 3 joints** | p=0.157 | p=0.675 | p=0.675 | p=0.432 | p=0.384 |
| **Morning stiffness** | p=0.566 | p=0.392 | p=0.836 | p=0.285 | p=0.802 |
| **Arthritis of hand joints** | p=0.157 | p=0.142 | p=0.529 | p=0.432 | p=0.562 |
| **Symmetric arthritis** | p=0.089 | p=0.955 | p=0.178 | p=0.675 | p=0.103 |
| **Rheumatoid nodules** | p=0.488 | p=0.529 | p=0.105 | p=0.736 | p=0.709 |
| **Radiographic changes** | p=0.925 | p=0.694 | p=0.670 | p=0.269 | p=0.928 |

Table XXXIII. Associations between ACR 1987 classification criteria and HLA-DRB1*04 sub-group alleles

| ACR 1987 criteria | *04:01 | *04:02 | *04:03 | *04:04 | *04:05 | *04:08 |
|---|---|---|---|---|---|---|
| **Arthritis ≥ 3 joints** | p=0.551 | p=0.551 | p=0.101 | p=0.423 | p=0.448 | p=0.551 |
| **Morning stiffness** | p=0.360 | p=0.423 | p=0.360 | p=0.930 | p=0.080 | p=0.360 |
| **Arthritis of hand joints** | - | - | - | - | - | - |
| **Symmetric arthritis** | p=0.423 | p=0.360 | p=0.423 | p=0.930 | p=0.770 | p=0.360 |
| **Rheumatoid nodules** | - | - | - | - | - | - |
| **Radiographic changes** | p=0.423 | p=0.360 | p=0.360 | p=0.930 | p=0.770 | p=0.423 |

If in case of ACR 1987 classification, association of each criterion did not revealed significant data, the correlation of HLA-DRB1 alleles with ACR/EULAR 2010 classification criteria showed promising associations like the correlation between potentially containing alleles and acute phase reactants (p=0,036), particularly of

*04:08 sub-group (p = 0.036, respective p = 0.011), but also between presence of *14 allele and positive serology for rheumatoid factor and/or anti-cyclic citrullinated antibodies, and supplementary with criterion score (p = 0.001).

Table XXXIV. Associations between ACR/EULAR 2010 classification criteria and potentially containing SE alleles

| ACR/EULAR 2010 Criteria | Potentially containing SE alleles | *04 | *01 | *10 | *14 |
|---|---|---|---|---|---|
| **Joint involvement** | p=0.491 | p=0.519 | p=0.268 | p=0.918 | p=0.301 |
| **Serology** | p=0.889 | p=0.0509 | p=0.277 | p=0.971 | p=0.001 |
| **Acute phase reactants (ESR;CRP)** | p=0.036 | p=0.890 | p=0.198 | p=0.491 | p=0.446 |
| **Duration of symptoms** | p=0.238 | p=0.518 | p=0.292 | p=0.799 | p=0.963 |

Table XXXV. Associations between ACR/EULAR 2010 classification criteria and HLA-DRB1*04 sub-group alleles

| ACR/EULAR 2010 Criteria | *04:01 | *04:02 | *04:03 | *04:04 | *04:05 | *04:08 |
|---|---|---|---|---|---|---|
| **Joint involvement** | p=0.642 | p=0.114 | p=0.642 | p=0.392 | p=0.937 | p=0.095 |
| **Serology** | p=0.466 | p=0.466 | p=0.224 | p=0.262 | p=0.679 | p=0.224 |
| **Acute phase reactants (ESR;CRP)** | p=0.695 | p=0.695 | p=0.695 | p=0.546 | p=0.617 | p=0.011 |
| **Duration of symptoms** | p=0.271 | p=0.463 | p=0.271 | p=0.259 | p=0.533 | p=0.463 |

Detailed analysis of positive serology which correlated globally with *04 allele, we observed a statistically significant association with anti-cyclic citrullinated peptides (p = 0.030), contrasting with rheumatoid factor (table XXXVI).

Table XXXVI. Associations between positive serology and HLA-DRB1*04 sub-group alleles

| | ***04** | ***04:01** | ***04:02** | ***04:03** | ***04:04** | ***04:05** | ***04:08** |
|---|---|---|---|---|---|---|---|
| **antiCCP** | p=0.030 | p=0.569 | p=0.326 | p=0.326 | p=0.279 | p=0.279 | p=0.326 |
| **RF** | p=0.318 | p=0.448 | p=0.448 | p=0.255 | p=0.243 | p=0.519 | p=0.255 |

Another important association was given by the disease activity score that correlated statistically significant with main potentially containing SE alleles (p=0.014), but also in particular with *04 or *14 alleles (table XXXVII). Not the same statistic significance was observed comparing with HLA-DRB1*04 sub-groups (table XXXVIII).

Table XXXVII. Associations between DAS28, radiographic changes and echographic changes and potentially containing SE alleles

| | **Potentially containing SE alleles** | ***04** | ***01** | ***10** | ***14** |
|---|---|---|---|---|---|
| **DAS28** | p=0.014 | p=0.052 | p=0.658 | p=0.258 | p=0.054 |
| **Radiographic changes** | p=0.999 | p=0.697 | p=0.999 | p=0.999 | p=0.545 |
| **Echographic changes** | p=0.960 | p=0.659 | p=0.392 | p=0.382 | p=0.519 |

Table XXXVIII. Associations between DAS28, radiographic changes and echographic changes and HLA-DRB1*04 sub-groups

| | ***04:01** | ***04:02** | ***04:03** | ***04:04** | ***04:05** | ***04:08** |
|---|---|---|---|---|---|---|
| **DAS28** | p=0.368 | p=0.999 | p=0.234 | p=0.695 | p=0.999 | p=0.865 |
| **Radiographic changes** | p=0.251 | p=0.154 | p=0.154 | p=0.621 | p=0.087 | p=0.251 |
| **Echographic changes** | p=0.919 | p=0.104 | p=0.919 | p=0.876 | p=0.605 | p=0.155 |

An important correlation was observed by analysing the association between potentially containing SE alleles and extraarticular manifestations in patients with early RA (table XXXIX). Globally, the best association was between *01 allele and chronic anaemia (p = 0,042), and in case of LA-DRB1*04 sub-grops, *0403 allele correlated with same manifestation (p = 0,008). *0405 allele presented a surprising correlation with pulmonary manifestations (p = 0,038) (table XL).

Table XXXIX. Association between potentially containing SE alleles and extraarticular manifestations in patients with early RA

| | **Potentially containing SE alleles** | ***04** | ***01** | ***10** | ***14** |
|---|---|---|---|---|---|
| Extra-articular manifestations | p=0.322 | p=0.304 | p=0.123 | p=0.999 | p=0.214 |
| Chronic anaemia | p=0.377 | p=0.213 | p=0.042 | - | p=0.928 |
| Pulmonary manifestations | p=0.421 | p=0.889 | p=0.889 | p=0.297 | - |
| Carpal tunnel syndrome | p=0.754 | p=0.307 | p=0.889 | p=0.297 | - |

Table XL. Association between HLA-DRB1*04 sub-groups and extraarticular manifestations in patients with early RA

| | ***04:01** | ***04:02** | ***04:03** | ***04:04** | ***04:05** | ***04:08** |
|---|---|---|---|---|---|---|
| Extra-articular manifestations | p=0.591 | p=0.283 | p=0.591 | p=0.680 | p=0.999 | p=0.591 |
| Chronic anaemia | - | - | p=0.008 | - | - | - |
| Pulmonary manifestations | - | - | - | - | p=0.038 | - |
| Carpal tunnel syndrome | p=0.101 | - | - | - | p=0.255 | - |

Related to the allele considered to be protective against rheumatoid arthritis HLA-DRB1*13, there was observed a powerful negative association with acute phase reactants globally (p = 0,016) and with the duration of symptoms (p = 0,017), which represent criteria in ACR/EULAR 2010 classification. Clasical criteria presented low

correlations, the only one that was statistically significant being the rheumatoid factor criterion (p = 0,052). There were no association with rheumatoid nodules (table XLI).

Table XLI – Associations between ACR 1987 - ACR/EULAR 2010 classification criteria and *13 allele

| **ACR 1987 classification criteria** | *13 allele | **ACR/EULAR 2010 classification criteria** | *13 allele |
|---|---|---|---|
| 1. Arthritis of 3 or more joint areas | p=0.562 | A. Joint involvement | p=0.679 |
| 2. Morning stiffness ≥ 60 min | p=0.802 | | |
| 3. Arthritis of hand joints | p=0.384 | | |
| 4. Symmetric arthritis | p=0.201 | B. Serology (FR, antiCCP global) | p=0,483 |
| 5. Rheumatoid nodules | - | | |
| 6. Serum rheumatoid factor | p=0.052 | C. Acute phase reactants (global) | p=0,016 |
| 7. Radiographic changes | p=0.146 | D. Duration of symptoms | p=0,017 |

Also, was important the therapeutically approach of patients with early RA that beneficiated by initiation of DMARDs therapy (DMARDs – disease modifying antirheumatic drugs) (table XLII). 6 denied or were unable to start therapy, receiving chronic gluco-corticoid treatment. Half of the patients received initially methotrexate, in a calculated average dose of 12 mg, 20,45% received treatment with leflunomide in an average dose of 20 mg, 15,90% treatment with cu hydroxycloroquine and only 6,82% therapy with cu sulphasalazine.

Table XLII. Initial DMARDs therapy in early RA patients

| **DMARDs** (disease modifying antirheumatic drugs) | | No. = 44 pac initiating DMARDs | Average dose (mg) | DMARDs per total ever (nr) |
|---|---|---|---|---|
| METHOTREXATE (nr., %) | | 22 (50%) | 12 mg | 24 |
| SULPHASALAZINE (nr., %) | | 3 (6,82%) | 2250 mg | 4 |
| HYDROXYCLOROQUINE (nr., %) | | 7 (15,90%) | 400 mg | 11 |
| LEFLUNOMIDE (nr., %) | | 9 (20,45%) | 20 mg | 15 |
| Combinations (nr., %) | | 3 (6,82%) | - | 6 |
| CYCLOPHOSPHAMIDE*(nr., %) | | 0 | - | 1 |
| AR at DMARDs (nr., %) | Total | 11 (25%) | | |
| | neurological | 1 | | |
| | digestive | 7 | | |
| | cutanate | 2 | | |
| | general | 3 | | |

*patients with associated demielynating pathology

Adverse reactions at DMARDs therapy were present in a procentage of 25%, with leading the digestive manifestations, and in a smaller range the general ones.

There were found no statistically significant correlations between adverse reactions of disease modifying therapy and potentially containing shared epitope alleles (table XLIII).

Table XLIII– Correlations between adverse reactions of disease modifying therapy and potentially containing shared epitope alleles

| | **Potentially containing SE alleles** | ***04** | ***01** | ***10** | ***14** |
|---|---|---|---|---|---|
| **Adverse reactions of DMARDs** | p=0.910 | p=0.791 | p=0.316 | p=0.377 | p=0.654 |

Anti-inflammatory treatment was cvasi-present in patients diagnosed with early RA (table XLIV), 84% using non-steroidal anti-inflammatory drugs and 72% treatment with corticosteroids. Adverse reaction percentage was low, both for NSAD and corticosteroid treatment (2%).

Tabelul XLIV. Steroidal and non-steroidal anti-inflammatory treatment in patients with early RA

| **Anti-inflammatory therapy** | **NSAD** | **corticosteroids** |
|---|---|---|
| Pac. and % from total | 42 (84%) | 36 (72%) |
| Adverse reactions | 1 (2%) | 1 (2%) |

# CONCLUSIONS

1. Rheumatoid arthritis represents the most frequent inflammatory rheumatic disease with a multi-factorial aetiology and auto-immune pathogenicity, mainly characterized by articular involvement with chronic, progressive, deforming evolution, but also by systemic and extra-articular manifestations, that implies a complex, individualized treatment represented by disease modifying drugs and biologic therapies.

2. Epidemiological trials analysis enlightens the dynamic character of the disease, with variations of incidence and prevalence that attests the need of close monitoring of cases.

3. The establishment of a complex, complete and very early diagnosis, that allows implementation of optimal therapies and an efficient follow-up over the evolution under treatment is of crucial importance in the assessment of patient in order to reduce the number of late stage, invalidating cases. The goal of the practitioner is obtaining remission, and ideally, the cure.

4. Having in mind that today there is no effective and certain way to diagnose early and very early rheumatoid arthritis, is imperative and justified the need of judicious classification criteria, and their identification represents a huge help in orienting clinical diagnosis.

5. Today, rheumatoid factor and anti-cyclic citrullinated antibodies are the accepted key elements both for the diagnosis of the disease, but also for predictive evaluation of functional and radiological outcome. The large range of sero-positive patients, either for rheumatoid factor, anti-cyclic citrullinated antibodies or both, seems to sustain enough the importance of serological markers into classification and diagnosis of early rheumatoid arthritis.

6. Biological syndrome of inflammation expressed by the acute phase reactants, especially erythrocyte sedimentation rate and C reactive protein must be evaluated for monitoring the evolution of disease and for predictind the functional and radiological damage. Increased values found in patients of our study correlates significantly with disease activity index DAS 28, but also with the presence of extra-articular manifestations.

7. The association with cardio-vascular diseases is definetly related with pro-inflammatory status and the wide range of cytokines involved into the pathogenesis of disease, but also with the use of cyclooxigenasis inhibitors. Compared with general population, mortality and morbidity by cardio-vascular causes are significanly increased in rheumatic patients and especially in rheumatoid arthritic cases, as it was demonstrated also by our results with a high procentage of this type of co-morbidities in the studied patients.

8. Rheumatoid arthritis is traditionally associated with other auto-immune diseases, but these were considered separate entities, especially because of different clinical manifestations (ex.tyroiditis). Data revealed in our study with top places occupied by cardiovascular associated disease and tyroidian associated disease shows the importance of the stratification of patients with RA into specific sub-groups.

9. Extra-articular manifestations were present in 30% of patients with early RA, with the highest incidence of chronic anaemia, compared with carpal tunnel syndrome or pulmonary manifestations, and the analysis of this particular subgroup revealed the importance of the presence of antibodies, but more of the titre of them, in the pathogeny of extraarticular manifestations in RA.

10. Imagistic assessment of early RA patients allowed the evaluation of structural changes by the use of articular x-rays and ultrasonography. Ultrasonography has a higher sensitivity for smooth tissue lesions, and magnetic resonance is considered to be elective in incipient erosions. Unfortunately, the last method is hardly used due to high costs.

11. Articular ultrasound exam reveals the "gold standard' in early rheumatoid arthritis diagnosis – synovitis, as shown by 82.6% from the total of patients performing the investigation that presented effusions/synovial proliferations, especially in hand joints.

12. The ultrasonographic expression of erosions was not important, partially due to the early moment of diagnosis, and the ratio of serologically positive patients with radiological changes was superior compared with the ratio of serologically positive patients with ultrasonographycal changes, showing the huge importance of the early diagnosis.

13. The analysis of ACR 1987 applied on early RA patients revealed the dominance of the criteria of hand arthritis, symmetric involvement and positive RF, compared with minimal range of presentation for radiological changes and rheumatoid nodules criteria.

14. The performance of ACR/EULAR 2010 classification criteria was remarkable, showing important associations stratified on each criterion with the disease activity parameters, underlining the importance of early identification of early RA patients and especially of those predisposed to destructive forms.

15. Many trials are announcing a high risk of developing the disease in serologically positive patients that associate smoking. Our very surprising conclusion of the study was the lack of statistical significance between the presence of anti-cyclic citrullinated peptide antibodies and smoking, antiCCPs being equally present in smokers and non-smokers, and RF had a similar trend with a small increase in non-smokers.

16. There were elaborated tables and algorithms that include the well-known theory of shared epitope, that managed to stratify the risk ratio for RA, but these cannot be applied as a rule for all groups, due to the wide variety of alleles that keeps this subject open, and still providing surprising correlations.

17. Existing studies assessed small population groups, with various ethnicities that must be integrated into a common database, especially because the pathogenic involvement seems to be more and more clearly defined by the citrullination of proteins in RA.

18. Genotyping the HLA-DRB1 alleles showed statistically significant differences both for potentially containing SE, and a number of other alleles. The most significant were the high frequencies for *04 and *10 (5% vs. 1.12%; $p = 0.0477$), but also for other alleles potentially containing SE like *01. HLA-DRB1*14 had a comparable frequency with control group. Very important seems to be the general distribution of alleles potentially containing SE, with a high frequency in early RA group. High, but not sufficiently statistic had a suspect allele *07.

19. With minor percentage differences, the total frequency of homozygots in RA group did not differ statistically significant compared with control group, with similar findings in case of homozygots potentially containing SE (*01, *04, *10, *14) or the carriage of two different alleles potentially containing SE.

20. The highest calculated relative risk was conferred by *10 allele – 4.63, followed by *04 (RR = 2.07), and the lowest for *01 and *14 with RR = 1.91,

respectively RR = 1.81; for the subgroups HLA-DRB1*04, the highest value was for *04:04 (RR = 4.34), compared with lower risks for *04:08 (RR = 2) or *04:01 and *04:05 (RR similar of 0.93).

21. From the potentially containing SE alleles, *04, *10 and *14 correlated very good with the presence of anti-citrullinated peptide antibodies, compared with *01. HLA-DRB1*14 correlated with the presence of rheumatoid factor.

22. More than a half of the smoker patients that presented alleles potentially containing SE were antiCCP positive, and 50% of them with high titres, even if the correlation between smoker status and sero-positivity was not statistically significant.

23. Serologically negative patients presented an interesting genetic pattern, with a high frequency for *01 allele, compared with *04 or *14, and the most intriguing aspect was the absence of *10 (which was generally the most frequent in early RA group), in serologically negative patients for antiCCP and in serologically negative for both patients, underlining a distinct phenotipical entity.

24. Correlating the genetic factors assumed also the inclusion of *13 allele, which presented a statistically significant negative association with early RA compared with control, but also for *15 with a low frequency in studied group and a negative association with the presence of rheumatoid factor.

25. The analysis of the phenotipical association of alleles and allelic subgroups found in our population with ACR1987 classification criteria did not revealed statistical significance, pursuing the definitive revision of the old classification set that represented the background for trials until now.

26. Our results in associating the new ACR/EULAR 2010 classification criteria with different alleles with potential were rough, but yet significant, and this sustains the need of a different, more comprehensive approach of the patient with RA.

27. Detailed analysis of positive serology correlated globally with *04 allele, showed a statistically significant correlation with anti-citrullinated peptides antibodies, in contrast with rheumatoid factor, but also a strong association with disease severity index DAS 28.

28. *13 allele, demonstrated to be protective, presented a powerful negative association with acute phase reactants and the duration of the disease, which represent criteria of ACR/EULAR 2010 classification.

29. Association of potentially containing SE alleles with extra-articular manifestations demonstrated in patients with early RA was dominated by the correlation between *01 and chronic anaemia, and for HLA-DRB1*04 subgroups, *0403 correlated with same manifestation (p = 0,008). Surprising was the correlation of *0405 with pulmonary manifestations.

30. The ground of therapeutically approach in rheumatoid arthritis patient is pharmacological treatment, with the goal of stopping the evolution of destructive lesions, reducing inflammation and pain, and implicitly the change in the quality of life. Initiation of a potential remissive therapy in the first year of evolution aims the "window of opportunity" that is hardly found in the absence of an early diagnosis.

31. The efficiency of new therapeutically strategies, including biological agents, on joint destructions, disease activity, functionality and quality of life has a profound social and economical impact. An efficient therapy can lead to reduction of direct and indirect costs given by the readjustment and even maintaining the work capacity in patients with RA.

32. The proper management is no longer an ideal goal, and the quality of life of the patients with this pathology can be improved by establishing an early clinical-

genetical-biological and imagistic diagnosis, that will allow a better integration and a reducement in the health costs. The absence of national trials and scientific research that may offer a genotipical pattern for rheumatoid arthritis patient and provide access to modern investigational tools represents a barrier in realizing this goal.

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