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PhD THESIS

- SUMMARY -

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VICTORIA - CRISTINA DUMINICĂ (ŞUŢA)

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PhD THESIS - SUMMARY

SPECIFIC FEATURES OF RHEUMATOID ARTHRITIS IN TATARS LIVING IN DOBROGEA AREA

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PhD Scientific Coordinator **Prof. Univ. Dr. ELVIRA CRAIU**

CONSTANŢA 2016

Motto:

„Nulla res me delectabit, licet sit eximia et salutaris,
quam mihi uni sciturus sum”
(Seneca, Epist.6,4)

„I might not be delighted with anything, even eminent and beneficial,
if I am the only one to know it.”
(Seneca, Epist 6, 4)

Keywords: rheumatoid arthritis, Caucasian population, Tatar population, epidemiology, etiology, socio-demographic features, clinical and biological characteristics

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I. Introduction

Rheumatoid arthritis is the most frequent inflammatory rheumatic disease, affecting between 0.5 and 1% of the general population. Left untreated or treated at a later stage, rheumatoid arthritis leads to early invalidity and social isolation, a significant socio-economic impact, and a reduced life expectancy by an average of 7 years.

The cause of the disease remains unknown; however, as with all autoimmune disorders, it is assumed that rheumatoid arthritis is the consequence of the influence of environmental factors on a genetically predisposed host. The role played by the genetic factor, represented by HLA and non-HLA genes, in the onset of rheumatoid arthritis is supported by the variation in disease prevalence amongst different ethnic groups. Regional variations in disease prevalence support the environmental influence theory: geographical and climatic conditions, industrialization and pollution, socioeconomic status or lifestyle. For the time being, with a few exceptions, such as smoking, the vast majority of factors that can influence the onset of the disease remain unclear, although their contribution is most probably significant.

Two different populations live in the Constanta County, under similar geographic, social, and economic condition: the majority Caucasian population and the Tatar ethnic minority of Mongolian descent. The clinical observations of the Rheumatology Department where I conduct my activity have suggested that rheumatoid arthritis seems to be more frequent and more severe in Tatar patients. These observations, and in particular those regarding disease prevalence, are surprising given the fact that Asian populations typically register low prevalence rates for rheumatoid arthritis.

The thesis aims to determine the epidemiological and clinical characteristics of rheumatoid arthritis amongst the two different ethnic population groups residing in the same geographical region. The results of our studies support the hypothesis of an increased prevalence of rheumatoid arthritis amongst the Tatar ethnic group and outline a series of disease particularities in this population; however, further observational epidemiological studies are required in order to confirm this data.

The study of a population with an increased risk for developing rheumatoid arthritis can shed light on the aetiology and thus offer primary prevention, early diagnosis and treatment solutions for this disease.

II. Objectives

1. Creating a database which includes patients with rheumatoid arthritis monitored by the most important clinical rheumatology department in Constanta County and analysing the demographic and clinical characteristics of these patients.
2. Analysing demographic and clinical characteristics of a subgroup of rheumatoid arthritis patients of Tatar ethnicity.

III. Materials and methods

Structuring of bibliographic database

In order to obtain bibliographic material, the main source of information was the World Wide Web, the following resources being consulted:

- **Medical information databases:**
  - UpToDate – www.uptodate.com
  - MedLine – PubMed – www.ncbi.nlm.gov
  - The Cochrane Library – www.thecochranelibrary.com
  - SpringerLink – www.springerlink.com;
  - Booksc - http://booksc.org
  - Scopus – www.scopus.com

- **Online specialty journals:**
  - Annals of the Rheumatic Diseases;
  - Arthritis and Rheumatism;
  - Rheumatology;
  - Clinical Rheumatology;
  - Arthritis Research & Therapy;
  - Journal of Rheumatology;
  - Rheumatology International;

- New England Journal of Medicine,
- European Heart Journal;
- The Journal of American College of Cardiology;
- The Journal of Immunology;

➢ **Medical content websites:**

- www.biomedcentral.com;
- www.nature.com;
- www.medscape.com;
- www.sciencedirect.com;
- www.sciencedaily.com;
- www.arthritis-research.com;
- www.findarticles.com;
- www.clinicaltrials.gov;

➢ **Specialty books:**

- Arthritis and Allied Conditions – a Textbook of Rheumatology, 15th ed., William J Koopman, Larry W Moreland;
- EULAR Textbook of Rheumatic Diseases;
- Esenţialul în reumatologie – Ruxandra Ionescu;
- Manualul Merck de diagnostic şi tratament, ed. XVII;
- Kelley's Textbook of Rheumatology, Saunders Company, Philadelphia, 7th ed.
- Ghidul serviciilor medicale al Laboratoarelor Synevo, ediţia 2007-2008

Patients selection

An observational longitudinal study which included 447 patients (380 females and 67 males) with ages between 18 and 86-years-old, admitted to the Rheumatology Department – Internal Medicine II Clinic of the Emergency Clinical County Hospital „Sf. Apostol Andrei" Constanta with the diagnosis of rheumatoid arthritis between January 2013 and December 2014. All patients expressed their agreement to participate in the study through a signed and dated informed consent at the time of admission. The

diagnosis of rheumatoid arthritis was established according to the ACR 1987 and ACR/EULAR 2010 criteria. Evaluation of all the patients was performed by the Department rheumatologist. Information obtained from the medical history, clinical examination, and paraclinical investigations (laboratory and imaging) were recorded at the same date. Patients under the age of 18 and those with a juvenile onset were excluded from the study. The Department's patients are monitored according to the treat-to-target strategy, with evaluations at every 1-3 months, depending on both disease activity and treatment regimen modifications.

The following **inclusion criteria** were used:

1. A diagnosis of rheumatoid arthritis according to the ACR 1987 and ACR/EULAR 2010 criteria (see annexes);

2. Age $\geq$ 18 years old;

3. Patient informed consent.

Exclusion criteria: cases of rheumatoid arthritis with juvenile onset.

The following data was recorded:

- Demographic data: gander, current age, age at onset, ethnicity (Tatar/Caucasian), area of residence (urban/rural), geoclimatic area of residence (Black Sea riverside area – continental Dobrogea), lifestyle (current smoking status, smoker at onset, smoker ever), body mass index (BMI), social status (legal worker, unemployed, age retirement, ill-health retirement, social welfare);
- Data regarding the history and characteristics of the disease: disease duration (mean duration, early disease, established disease), age at onset, interval between onset and diagnosis and evolution during the past 15 years (2000 – 2014) of rheumatoid arthritis incident cases registered by the rheumatology department, type of medical specialist who established the diagnosis, disease type (seropositive or seronegative), the presence of bone erosions, degree of disease activity (analyzed accoding to DAS28-ESR, DAS28-CRP, CDAI, SDAI), complications (arthroplasty), treatment – conventional DMARDs, biological DMARDs, glucocorticosetoides, NSAIDs.

- Patient evaluations: height, weight, BMI, BP, tender joint count, swollen joint count, patient global assessment and physician global assessment, presence of ankylosis, presence of extra-articular manifestations at the time of examination or in the patient's medical history, comorbidities.
- Biologic modifications: rheumatoid factor (latex), antiCCP antibodies, acute phase reactants (erythrocyte sedimentation rate – ESR, C reactive protein - CRP), complete blood count, glucose levels, lipid profile (total serum cholesterol, HDL-cholesterol, triglycerides), alanine transaminase (ALAT) and aspartate aminotransferase (ASAT).

IV. Statistical Analysis

Experimental data was processed with the use of the statistical analysis software *IBM SPSS Statistics 20.* The following procedures were used:

- *Descriptive statistics* (determination of *absolute frequencies* and *percentages* corresponding to categorical variables, calculation of *statistical indexes,* of central tendency, dispersion, asymmetry and vaulting, *verifying the existence of potential outliers,* and, on a case-by-case basis, eliminating/replacing them with recalculations of the concerned indexes, *verifying the normality condition* for continuous variables).
- *Graphics* (Bar, Pie, Histogram, Error Bar, Bar+Error Bar, Scatter).
- *Parametric statistical tests* (T-test for comparing a sample mean to a specified value, T-test for comparing the mean of two independent/dependent means, ANOVA One-Way test).
- *Nonparametric statistical tests* (χ^2 association test, of the relationship between two categorical variables, with the determination in certain circumstances of the OR opportunity-risk ratio and Rr relative risk).
- *Correlation analysis* (performed both for variables measured at an interval/ratio level and for variables at a nominal and ordinal level).
- *Logistic regression analysis* (used to establish the relationship between a sum of categorical/continuous *independent variables* and a nominal/binary *dichotomous dependent variable*, whose values are codified 0/1).

V. Results

V.a. Study I results

The study included 447 patients with adult-onset rheumatoid arthritis (≥ 18 years), 380 women (85%) and 67 men (17%), accounting for a female:male ratio (F:M) of almost 6:1 (5.7:1) (Table 1).

Table 1. Demographic characteristics of RA patients(n= 447)

Variable	**Number (%)**	**Mean value and standard deviation**
Current age (years)		62.13 ±11.44
Age at onset (years)		51.71 ± 13.67
Women	380 (85%)	
Caucasian	395 (88.4%)	
Urban	357 (79.86%)	
Riverside area	319 (71.36%)	
Non-smokers	300 (67.10%)	
Employees	91 (29.35%)	
Ill-health retirement	115 (25.72%)	
Social welfare	190 (42.50%)	
BMI (Kg/m²)		27.19± 5.17

The F:M ratio modifies according to the demographic characteristics of the patients. As it is outlined by the subsequent statistical analysis of the sample population, the F:M ratio registers important variations according to current age and age at onset, according to smoking status at onset or in the time frame preceding the onset, according to the area of residence, or patient BMI.

Age at onset appears to be influenced by gender, smoking, and the presence of anti-CCP antibodies. Logistic regression analysis of the influence of demographic factors (gender and smoking) and of the presence of anti-CCP antibodies at the age of onset shows that the risk for early onset (under 52 years old, which represents the average for the sample population) is 3.109 times higher for women (Wald1 = 13.521, df = 1, $p < 0.001 < \alpha = 0.05$. ExpValue(B1) = 3.109), 2.827 times higher for smokers ($Wald_2$ = 15.798, df = 1, $p < 0.001 < \alpha = 0.05$. ExpValue($B_2$) = 2.827) and 2 times higher for

patients with positive anti-CCP (Wald3 = 9.662, df = 1, p = 0.002 < α = 0.05. ExpValue(B3) = 1.990). The global analysis for the risk of early onset (<52 years) shows an OR=3.553 for the female patient, smoker at disease onset, and positive for anti-CCP antibodies.

Among disease characteristics, the following were included: disease type (early-onset, late-onset, early RA, established RA, seropositive or seronegative), clinical manifestations (musculoskeletal and extra-articular) present at the time of examination or in the patient's medical history, laboratory findings (rheumatoid factor and anti-CCP antibodies analysis, analysis of acute phase reactants), the degree of disease activity (evaluated according to DAS28-CRP, DAS28-ESR, CDAI, SDAI), complications (arthroplasty), cardiovascular risk, comorbidities, and treatment (Table 8).

Table 8. Clinical characteristics of RA patients

Characteristics	**Number (sample population: 447)**	**Percentage**	**Mean, mean standard deviation**
Disease duration (years)			10.75±8.85 (9)
Onset-diagnosis interval (months)			16.3±24.1 (6)
Onset-diagnosis interval for early disease (months)	56		4.09±3.73 (3)
RF (+)	359	80.3%	
Anti-CCP (+)	300 (din 425)	67.1%	
Extra-articular manifestations	268	60%	
Erosions	337	75.4%	
DAS28 ESR			4.78±1.61 (4.72)
Remission and low activity	81	18.1%	
Conventional DMARD	344	77%	
Biologic therapy	103	23%	
Current corticotherapy	193	43.2%	

Our Rheumatology Department, established in the year 2000, treats and monitors patients with known and pre-existing disease prior to 2000 and patients whose diagnosis was established after 2000. In our sample population, 77.9% of patients (348 new patients over the span of 14 years) received the diagnosis during and after 2000, which amounts to an average of 25 new cases per year in the largest specialty department in the Constanta County. The number of new cases of rheumatoid arthritis is relatively constant between

2000 and 2014 (Figure 11). Following the introduction of the new ACR/EULAR 2010 classification criteria, no increase in the number of newly diagnosed cases was registered.

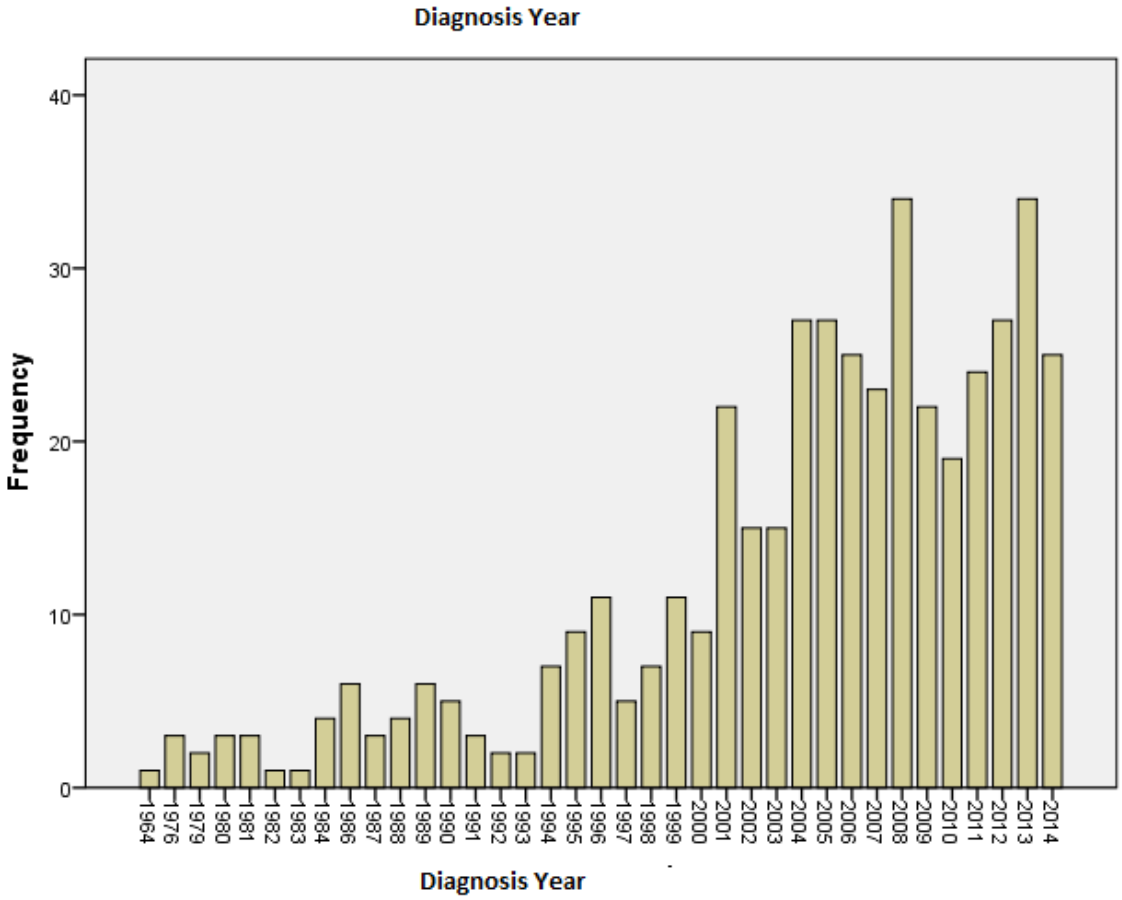

Figure 11. Distribution of RA cases according to the year of diagnosis

Rheumatoid factor (RF) was present in 80.3% of the patients from our sample population: 77.6% males and 80.8% females, without any significant differences in what regards relation to gender (Table 9). From the subsample of patients with a positive RF, 76.3% registered high values (>3N), also without significant differences between the two genders. Anti-CCP antibodies were determined quantitatively in 96.6% of the 447 patients included in the study. High titres (>3N) were registered in 91% of the patients positive for anti-CCP antibodies. The presence of the rheumatoid factor is associated in most cases with the presence of anti-CCP antibodies: out of the 346 RF (+) patients in which the presence of anti-CCP antibodies was evaluated, 292 (81.33%) were positive for

both antibodies (p<0.001). RF is present in 40.9% patients with negative anti-CCP antibodies, while anti-CCP antibodies are present in the absence of the RF in only 9% of cases. The presence of the RF is associated with smoking status at disease onset (p=0.003) (OR =2.767; 95%CI: 1.375 – 5.567), with the presence of erosions (p=0.001)(OR =2.345; 95%CI: 1.425 – 3.859) and ankyloses (p<0.001) (OR=3.467; 95%CI: 1.817 – 6.615). The presence of the rheumatoid factor is also associated with the presence of extra-articular manifestations (p<0.001) (OR=3.572; 95%CI: 2.191-5.824): subcutaneous rheumatoid nodules (p<0.001) (OR=6.862; 95%CI: 2.705-17.405) and simple chronic anaemia (p=0.001) (OR=2.547; 95%CI: 1.470-4.413). The presence of anti-CCP antibodies is correlated with smoking status, regardless of the period (p=0.006), smoker at onset (p=0.001), the presence of erosions (p<0.001), ankyloses (p<0,001), the presence of extra-articular manifestations (p<0.001) – rheumatoid nodules (p<0.001), simple chronic anaemia (p=0.004), and anti-CD20 therapy (p=0.004).

In what regards musculoskeletal manifestations, their evaluation was limited to identifying patients with joint ankylosis, tender and swollen joints count (the 28 included in the DAS score) and patient self-evaluation (patient global activity) (Table 11).

Table 11.Musculoskeletal manifestations of RA patients

	N	Minimum	Maximum	Mean	Std. Deviation
P-VAS [mm]	447	,00	100.00	53.8613	25.93375
E-VAS[mm]	447	,00	100.00	49.3512	23.83905
TJC	447	,00	28.00	8.1723	7.82025
SJC	447	,00	27.00	2.6957	3.98951
Valid N (listwise)	447				

Extra-articular manifestations are present in 268 patients (60%), with similar percentages in both sexes (p=ns). According to frequency, the following extra-articular manifestations were present in the sample population:

- Simple chronic anaemia (normocytic normosideremic anaemia) in 37.4% of cases (167 patients), more frequent in women than in men (p=0.05);

- Rheumatoid nodules in 24.6% of cases (110 patients), without significant differences amongst the two sexes (p=ns);
- Pleuropulmonary manifestations in 10% of cases (45 patients): 27 patients (6%) with interstitial pulmonary disease (confirmed through DLCO and pulmonary CT-scan), 10 patients (2.2%) with rheumatoid nodules and 8 patients (1.8%) with pleurisy;
- Ophthalmological manifestations in 3.6% of cases (16 patients): 11 patients (2.5%) with xerophthalmia and 5 patients (1.1%) with episcleritis. Episcleritis is more frequent in men (2 men out of 67 and 3 women out 380), while sicca syndrome is more frequent in women;
- Rheumatoid vasculitis in 3.8% of cases (17 patients): 8 patients (1.8%) with cutaneous vasculitis and 9 patients (2%) cu distal symmetric polyneuropathy through vasculitis of the vasa nervorum;
- Pericarditis in 1.3% of cases (6 patients);
- Felty syndrome in 0.2% of cases (one patient).

Erosions were present in 337 out of the total of 447 patients (75.4%): 45 out of 67 men (67.2%) and 292 out of 380 women (76.8%), but variations according to gender did not reach the statistical difference threshold (p=0.09). Patients with erosions have an average age of 62.42±10.96 years, with a minimum of 26 and maximum of 86 years, with an average disease duration of 13.12±8.69 years, with a minimum of several months (< 12 months) and a maximum of 56 years, an average age at onset of 49.67±13.00 years and an interval between onset and diagnosis with an average length of 18.25±25.73 l months (with a high standard deviation). Patients without erosions typically exhibit late onset (p<0.001), a shorter disease duration (p<0.001) and a shorter interval between onset and diagnosis (p=0.001) (Table 13).

Table 13. Characteristics of RA patients with bone erosions

Variables	**Erosions (+) (337 patients)**	**Erosions(–) (110 patients)**	**P**
Current age (years)	62.42 (10.93)	61.24 (12.83)	Ns
Age at onset (years)	49.67 (13.00)	57.95 (13.85)	<0.001
Interval between onset and diagnosis (years)	18.25 (25.73)	9.61 (17.19)	<0.001
Disease duration	13.12 (8.69)	3.47 (4.08)	<0.001

The patients in our sample population registered an average C-reactive protein value of 1.08mg±1.35 mg/dl (0.01 -12.04 mg/dl) (mean of 0.56 mg/dl) as opposed to a maximum normal value of 0.5mg/dl. 48% patients presented with normal CRP values (≤ 0.5mg/dl). Values of ≤1mg/dl, compatible with remission were present in 66.6% of patients. CRP values do not vary according gender, ethnicity, current smoker status, or body mass index. Average CRP values are higher in seropositive disease with high antibody titres and in erosive disease, but the registered differences are small and do not reach the statistical significance threshold. In our sample population, the average ESR value is 36.35±24.21 mm/h, with significant differences between extreme values (4-125 mm/h) and a mean value of 32 mm/h. A significant percentage of our sample population (194 patients: 43.4%) registered normal ESR value (≤28 mm/h), with a mean value of 16.47±6.11 mm/h, with extreme values within the normal range (4 and 26 mm/h) and a mean of 17mm/h. The average ESR value is strongly correlated with all composite scores ($p<0.001$) and with each element of this score (patient global assessment, tender joints count, swollen joints count) ($p<0.001$) with the exception of C-reactive protein ($p=0.455$).

Increases in C-reactive protein values do not determine a change neither of the disease activity interval nor of the average values of the clinical elements of the scores. On the other hand, an increase in ESR values (>28mm/h) changes the degree of disease activity, regardless of CRP values, also leading to a visible increase of the clinical elements of the utilized composite scores (Table 14).

Table 14. Acute phase reactants and disease activity

Variables	**ESR (-) CRP (-)**	**ESR (+) CRP (-)**	**ESR (-) CRP (+)**	**ESR (+) CRP (+)**
No. (%) out 447	94 (21%)	121 (27%)	108 (24.1%)	128 (28.6%)
P-VAS	46.6±22.4	59.0±26.7	46.4±23.9	60.6±27.0
E- VAS	40.4±19	56.2±24.4	40.7±20.4	56.7±25.0
TJC	6.7±6.4	9.5±8.5	6.3±7.5	9.5±7.8
SJC	1.5±2.4	3.6±4.7	1.7±3.3	3.4±4.2
ESR	17.7±6.6	50.8±21.5	16.1±6.1	53.3±21.7
CRP	0.2±0.1	0.2±0.1	1.8±1.2	1.9±1.6
DAS28 ESR	4.0±1.2	5.4±1.5	3.8±1.3	5.4±1.5

DAS28 CRP	3.3±1.2	4.1±1.5	3.7±1.3	4.5±1.4
CDAI	17.4±12.5	24.7±16.0	16.8±13.2	24.9±14.9
SDAI	17.8±12.6	24.9±16	18.6±13.2	26.8±15.2

Degree of disease activity and percentage of remission varies according to the evaluation score used (Figure 13).

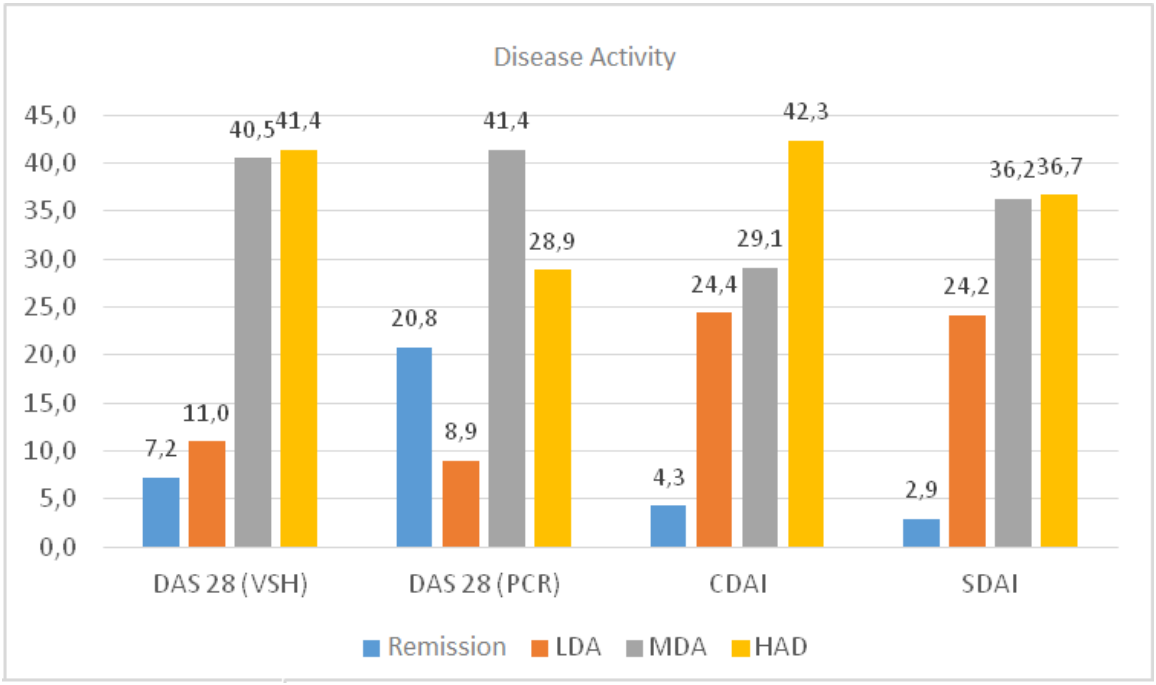

Figure 13. Case distribution (%) according to degree of disease activity, using various composite scores

Remission and low activity are registered in a percentage that varies between 18.1% and 29.8%. The lowest rate of remission and low activity is registered through the DAS28 ESR evaluation (18.1%). The other three score outline similar percentages for this group of patients: 27.1% (SDAI), 28.7% (CDAI), and 29.7% (DAS28 CRP) (Figure 13). Low activity disease is characterized in our study by a small number of swollen joints (<1), medium-normal ESR values (<30mm/h) and CRP values <1mg/dl (with the exception of SDAI). Consequently, patients with low activity on the aforementioned scores meet remission criteria according to the Booleane definition (Table 15). The classification of patients as having either remission or low activity disease, regardless of

the composite score used, is exclusively conditioned by subjective assessment components: P-VAS, E-VAS, TJC (Table 15).

Table 15. Low activity according to the elements of the 4 scores used

Low activity	**No.**	**TJC**	**SJC**	**E-VAS**	**P-VAS**	**ESR**	**CRP**
DAS28 ESR [2.6-3.2)	49	1.04±1.56 (00 -07)	0.14±,0.61 (00 -04)	24.08±10.78 (10-50)	27.55±15.17 (10-70)	26.16±15.80 (10-50)	0.83±0.87 (0.1 – 3.50)
DAS28 CRP [2.6 – 3.2)	40	2.25±1.46 (00 -06)	0.62±1.07 (00 -04)	34.25 ±9.64 (20-55)	39.37 ±13.54 (20-70)	26.25±16.03 (05-73)	0.76±0.97 (0.3 – 4.79)
SDAI [3.4 - 11]	108	1.21±1.16 (00-04)	0.13±0.39 (00-02)	24.30±8.87 (10-50)	26.20±10.95 (10-50)	29.65±18.31 (05-99)	1.22±1.62 (0.1-4.79)
CDAI (2.8 – 10]	109	1.35±1.19 (00-05)	0.17±0.46 (00-02)	25.50±8.49 (10-50)	27.20±10.14 (10-50)	29.71±18.53 (05-99)	0.88±1.03 (0.1-5.35)

Regardless of the score used to evaluate disease activity, over a quarter of patients exhibit high disease activity, with percentages that vary between 28.9% and 42.3% of the patients in the study.

Table 16. High disease activity according to the components of the 4 scores used

High activity	**No. (%)**	**TJC**	**SJC**	**E-VAS**	**P-VAS**	**ESR**	**CRP**
DAS28 – ESR > 5,1	185 (41.4%)	14.98±6.94 (03 -28)	5.56±4.77 (00 -27)	71.32±15.49 (25-100)	75.97±16.98 (40-100)	48.87±25.57 (7-125)	1.11±1.49 0.2 – 12.04)
DAS28 – CRP > 5,1	129 (28.9%)	17.22±6.76 (03 -28)	6.47±5.16 (00 -27)	74.10 ±16.21 (25-100)	79.84 ±16.17 (40-100)	48.20±27.21 (7-125)	1.46±1.74 (0.2 – 12.4)
SDAI >26	164 (36.7%)	16.27±6.57 (03-28)	5.89±4.94 (00-27)	71.73±16.54 (20-100)	76.76±17.48 (20-100)	46.24±26.60 (7-125)	1.22±1.62 (0.2-12.04)
CDAI >22	189 (42.3%)	15.38±6.65 (03-28)	5.45±4.79 (00-27)	70.10±16.70 (20-100)	75.19±17.40 (20-100)	45.52±26.71 (7-125)	1.14±1.53 (0.2-12.04)

The lowest rate of active disease is registered through the DAS28 CRP evaluation, while the DAS 28 ESR and CDAI scores contribute almost equally to the highest rate (Figure 13). The mean values of activity score components are correlated with high disease activity (Table 16). The individual analysis of objective components in the composite scores used to evaluate disease activity outline discrepancies between them and the global score. Consequently, there is a significant percentage of patients with high disease activity without swollen joints (between 8.9% and 20.7%), with normal ESH values (between 22.8% and 34.4%) or with normal CRP values (between 44.6-49.5%) (Table 17).

Table 17. High disease activity and objective elements of the composite scores

HIGH ACTIVITY	**SJC**		**ESR**		**CRP**	
	0	≥6	<30mm/h	≥30mm/h	<0,5 mg/dl	≥3xVN
DAS28ESR > 5.1	13.8%	45.2%	26.6%	73.4%	47.3%	22.9%
DAS28CRP > 5.1	8.9%	62.4%	22.8%	77.2%	44.6%	23.8%
SDAI > 26	20.7	37.4%	32.6%	67.3%	46.3%	25.6%
CDAI > 22	17.2%	44.3%	34.4%	65.6	49.5%	24.4%

The number of patients with high disease activity is significantly higher than that of those who have reached the therapeutic target (remission and low disease activity). Increased percentages of patients with high disease activity can be observed in cases with high RF titres (>3N) and in patients with simple chronic anaemia. Patients with high disease activity receive glucocorticosteroids in a higher percentage and the rate of those under biologic therapy with anti-TNF-alpha agents is lower.

All patients are under disease modifying therapy: conventional disease modifying therapy (monotherapy or combined therapy) in a percentage of 77% (344 patients) and biologic disease modifying therapy in a percentage of 23% (103 patients). Current conventional disease modifying therapy is represented by Methotrexate (MTX) in 61.5% cases, Leflunomide (LEF) in 30.4% of cases, Sulfasalazine (SSZ) in 25.7% and Hidroxicloroquine (HCQ) in 22.6% of cases. 70% of patients (240) with conventional therapy are under monotherapy, while the remainder of 30% (104 patients) are under combined therapy. The most frequent combinations are represented by triple therapy (MTX+SSZ+HCQ) in 35 cases (33.6% of combinations using conventional medication) and LEF+SSZ in 31 cases (29.52%).

Biological therapy is represented by anti-TNF-alpha agents administered to 11.25% of patients (50 patients), anti-CD20 agents administered to 11.6% of patients (52 patients) and tocilizumab (3 patients). In all cases, biological therapy is associated with a conventional drug, the most frequently associated conventional DMARD being MTX (56% of cases). Anti-TNF-alpha agents are associated with MTX in 32 cases (64% - 8 cases of monotherapy with MTX and 17 cases of triple therapy with MTX) and with LEF in 12 cases (24%). Rituximab is equally associated with MTX (25 cases) and LEF (25 cases).

Out of the 50 patients under treatment with anti-TNF-alpha agents, 32% have reached the therapeutic target (DAS28 ESR): 14% (7 patients) in remission and 18% (9 patients) with low disease activity (Table 19). A significant number of patients treated with anti-TNF-alpha agents exhibit high disease activity (26%). Current smoker status does not influence response to anti-TNF-alpha therapy. Patients treated with anti-TNF-alpha agents present a higher rate of erosions (49 out of 50 patients), extra-articular manifestations (41 out of 50 patients), primarily represented by subcutaneous rheumatoid nodules (24 out of 50 cases) compared to the remained of the sample population. There is no correlation between the use of anti-TNF-alpha agents and the presence of the rheumatoid factor or of the anti-CCP antibodies.

Table 19. DAS28 ESR intervals in RA patients receiving anti-TNF-alpha therapy

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Remission	7	14.0	14.0	14.0
	LDA	9	18.0	18.0	32.0
	MDA	21	42.0	42.0	74.0
	HDA	13	26.0	26.0	100.0
	Total	50	100.0	100.0	

Out of the 52 patients receiving rituximab, 19.2% have reached the therapeutic target (DAS2 ESR): 7.7% (4 patients) in remission and 11.5% (6 patients) with low disease activity (Table 20).

Table 20. DAS28 ESR intervals in RA patients receiving anti-CD20 agents

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Remission	4	7.7	7.7	7.7
	LDA	6	11.5	11.5	19.2
	MDA	22	42.3	42.3	61.5
	HDA	20	38.5	38.5	100.0
	Total	52	100.0	100.0	

Patients treated with anti-CD20 agents exhibit inflammatory anaemia ($p=0.033$), erosions ($p<0.001$), ankyloses ($p=0.005$) and seropositive disease characterised by the

presence of anti-CCP antibodies (p=0.005) or rheumatoid factor (p=0.011) more frequently. A significant percent (38.5%) are patients with active disease (Table 20). Prednisone was initially administered in combination with DMARDs in 75.4%, was ever administered in 84.8% cases and is currently administered to 43.2% of the patients in the study. In case of early disease, prednisone was initially administered in 85.7% of cases and is currently administered (throughout an evolution of ≤ 12 months) in 69.6% of cases. Ever administered corticotherapy is highly associated with vertebral fractures (p=0.036), but not significantly correlated with non-vertebral fractures, aseptic osteonecrosis, diabetes mellitus, or arterial hypertension.

77 vertebral fractures (16.9%) and 52 non-vertebral fractures were registered. Table 21 outlines risk factors for vertebral fractures for the RA patients in our study.

Table 21. Risk factors for vertebral fractures in RA patients

	Vertebral fracture (+)	**Vertebral fracture (-)**	**P**	**OR**
No (%)	75	372		
Female	68 (90.7%)	312 (83.9%)	Ns	
Caucasian	65 (86.7%)	330 (88.7%)	Ns	
Riverside area	52 (69.3%)	267 (71.8%)	Ns	
Urban	60 (80%)	297 (79.8%)	Ns	
Average age	70.89±8.03	60.36±11.22	<0.001	
Age at onset	56/33±13.92	50.77±13.45	0.001	
Disease duration	14.68±9.09	9.96±8.60	<0.001	
Early disease	4 (5.3%)	52 (14%)	0.037	
Eora	51 (68%)	166 (44.6%)	<0.001	OR=2.63
Smoker ever	19 (25.3%)	128 (34.4%)	Ns	
BMI	26.45±5.61	27.34±5.08	Ns	
Underweight	3 (4%)	8 (2.2%)	-	
Obese	14 (18.7%)	96 (25.8%)	-	
RF (+)	62 (82/7%)	297 (79.8%)	Ns	
Anti CCP (+)	48 (64%)	252 (67.7%)	Ns	
Erosions	69 (92%)	268 (72.0)	<0.001	OR=4.46
Ankyloses	43 (57.3%)	96 (25.8%)	<0.001	OR=3.86
Extra-articular manifestations	56 (74.7%)	212 (57%)	0.004	OR=2.22
Chronic anaemia	39 (52%)	128 (34.4%)	0.006	OR=2.06
Rheumatoid nodules	26 (34.7%)	84 (22.6%)	0.039	OR+1.81
Nonvertebral fractures	21 (28%)	31 (8.3%)	<0.001	OR=4.27
Aseptic osteonecrosis	3 (4%)	18 (4.8%)	Ns	
ESR	42.20±26.23	35.17±26.64	0.022	
CRP	1.27±1.83	1.05±1.23	Ns	
SJC	3.10±5.13	2.61±3.72	Ns	
TJC	8.60±8.40	8.08±7.70	Ns	
P-VAS	59.33±26.41	52.75±25.73	Ns	
E-VAS	54.00±25.12	48.41±23.49	Ns	
DAS28 ESR	4.96±1.85	4.75±1.55	Ns	
High activity	33 (44%)	152 (40.9%)	-	
Prednisone ever	69 (92%)	310 (83.3%)	0.057	

52 patients have nonvertebral fractures: 8 men and 44 women (p=0.932). With one exception, all nonvertebral fractures in female RA patients appear in postmenopause. Similar to vertebral fractures, nonvertebral fractures are not correlated with other risk factors, such as smoking, BMI, or corticotherapy. Moreover, no significant statistical correlation with the area of residence is noted. Nonvertebral fractures are statistically linked with the presence of extra-articular manifestations (p=.0.01; OR=2.442), erosions (p=0.006; OR=3.403), and ankyloses (p=0.016; OR=2.087).

We identified 21 cases of aseptic osteonecrosis (4.7%) – 5 men and 16 women – with a mean age of 61.52 ±12.44 years and a mean disease duration of 13.24±9.13 years. In 61.6% of cases, the disease had an early onset (prior to 52-year-old, which is the mean of the study), at a mean age of 48.24±13.86 years. The presence of aseptic osteonecrosis establishes significant statistical correlations with erosive disease (p=0.035), with certain comorbidities such as malignant neoplasia (p=0.009; OR=6.44) or with certain complications such as joint prosthetics (p<0.001; OR=23.21).

A small percent of the patients in the sample population (4.3%) underwent surgical interventions for joint prosthetics. Artroplasty is directly and statistically significantly correlated with the presence of aseptic osteonecrosis, 9 out of the 24 patients with prosthetics also having the diagnosis of aseptic osteonecrosis (p<0.001).

The following comorbidities were noted: obesity, diabetes mellitus, cardiovascular disease (HTN, ischemic coronary disease) and severe cardiovascular events (myocardial infarction and cerebral stroke), neoplasia, hepatitis B or C viral infections.

In our sample population, 43.8% (196 patients) of cases have an increased cardiovascular risk (over 5%). They exhibit an average SCORE value of 14.39±11.24 with a mean value of 10.50. Patients with rheumatoid arthritis and increased cardiovascular risk are women in a percentage of 76.5%, with a mean age of 71.13±6.71 years, late onset (at a mean age of 60.30±11.69 years), are active smokers or have smoked at one point in 13.3% and 32.1% of cases respectively, and have a mean disease duration of 11.17±9.49 years. 78.6% suffer from HTN, 26% from diabetes mellitus and they have a mean total cholesterol value of 214.56± 49.41 mg/dl. Almost all patients have an increased cardiovascular risk and HTN for which they receive antihypertensive

treatment. On the other hand, out of the patients with dyslipidemia and increased cardiovascular risk, only 33% are under statin treatment. In the RA patients with increased cardiovascular risk (SCORE>5%), SCORE values are strongly and significantly influenced by current age (mean>70 years old), late onset (mean>60 years-old) and male gender. In this sample of patients, a significant correlation between SCORE values and inflammation markers was not outlined.

In what regards malignancies, our sample population registers 20 cases of cancer (4.5%), out of which 17 (3.7%) are represented by solid neoplasms. No cutaneous neoplasia (melanoma or basal cell carcinoma) is registered, but a wide variety of solid neoplasms localisation is noted (thyroid – 3 cases, kidney – 3 cases, breast – 2 cases, prostate gland – 2 cases, lungs, cavum, laryinx, ovary, liver, colon, and uterus – 1 case each). The most frequent cancers (thyroid and kidney) were noted in female RA patients. 3 cases of haematological malignancies were also noted: one case of chronic lymphatic leukaemia and 2 cases of non-Hodgkin lymphoma, all of them being females and one of them is under anti-TNF-alpha therapy for 10 years.

In what regards hepatitis C viral infection, all patients with positive anti HVC antibodies were reported, accounting for a percent of 6.9% of the sample population. Viral RNA was measured in only 18 of the 31 patients with positive antibodies and it was detectable in half of the cases. To sum up, 9 patients have positive anti HVC antibodies with positive HVC RNA, 9 have positive antibodies without HVC RNA (disproving a chronic hepatitis C infection), with the remainder of 13 patients with positive anti HVC antibodies never being tested for HVC RNA. Hepatitis B viral infections only included patients with positive HBsAg. Testing for occult HBV infections was performed only in small number of cases. In our sample population, the percentage of RA patients and positive HBsAg is 3.8%.

V.b. Study II results

Constanta county is home to the largest Tatar ethnic group (24194 Tatars according to the last census), amounting to 3.3% of the county's population. In the analysed RA sample population, 52 of the patients are of Tatar ethnicity (11.6%): 9 males (13.4% of the male patients in the sample population) and 43 females (11.3% of the

female patients included in the study), amounting to a F:M ratio > 4:1 (4.77:1). A comparison of the two percentages (that of Tatars in the county's population and that of Tatars compared to the patients in the study) strongly supports the hypothesis that RA has an increased prevalence amongst Tatar ethnic groups (Difference = 0.16%, 95% CI = -1.092 to 1.41, Chi-square = 5.627, df = 1, p = 0.0177 < 0.05). The mean age of Tatar RA patients is 61.27±10.74 years with a mean age at onset of 49±15.12 years (between 19 – 79-years-old) (mean age 49-years-old). The mean age at onset for women is 48.07±15.79 years, while the mean age at onset for Tatar males is 53.44±11.05 years. There is a downward trend in what regards age at onset for Tatar ethnics (Figure 1), a smaller percentage of RA patients of Tatar ethnicity with late onset being noted (over 52 years old, which is the mean age at onset for the entire sample population in Constanta county) (Table 1).

Table 1. Demographic characteristics of Tatar RA patients

	Caucasians	Tatars	P
NO.	**395**	**52**	**7:1**
Sex	337 (85.3)	43 (82.7%)	Ns
Current age	62.24±11.5	61.27±10.7	Ns
Age at onset	52.06±13.45	49.00±15.12	Ns
Onset>52 (late onset)	197 (49.9%)	20 (38.5%)	***0.08***
Urban residence	313 (79.3%)	44 (84.6%)	Ns
Riverside area	280 (70.9%)	39 (75%)	Ns
Smoker ever	129 (32.7%)	18 (34.6%)	Ns
Smoker current	15 (12.9%)	8 (15.4%)	Ns
BMI	27.26±5.17	26.6±5.2	Ns
Employees	83 (21%)	8 (15.4%)	***0.012***
Ill-health retirement	92 (23,3%)	23 (44.2%)	***0.012***
Social welfare	160 (40.5%)	30 (57.7%)	***0.018***

Significant differences are not noted between the characteristics of rheumatoid arthritis in Tatar patients compared to Caucasian patients: a higher anti-CCP antibody titre mean and more frequent extra-articular manifestations, in particular simple chronic anaemia (Figure 2).

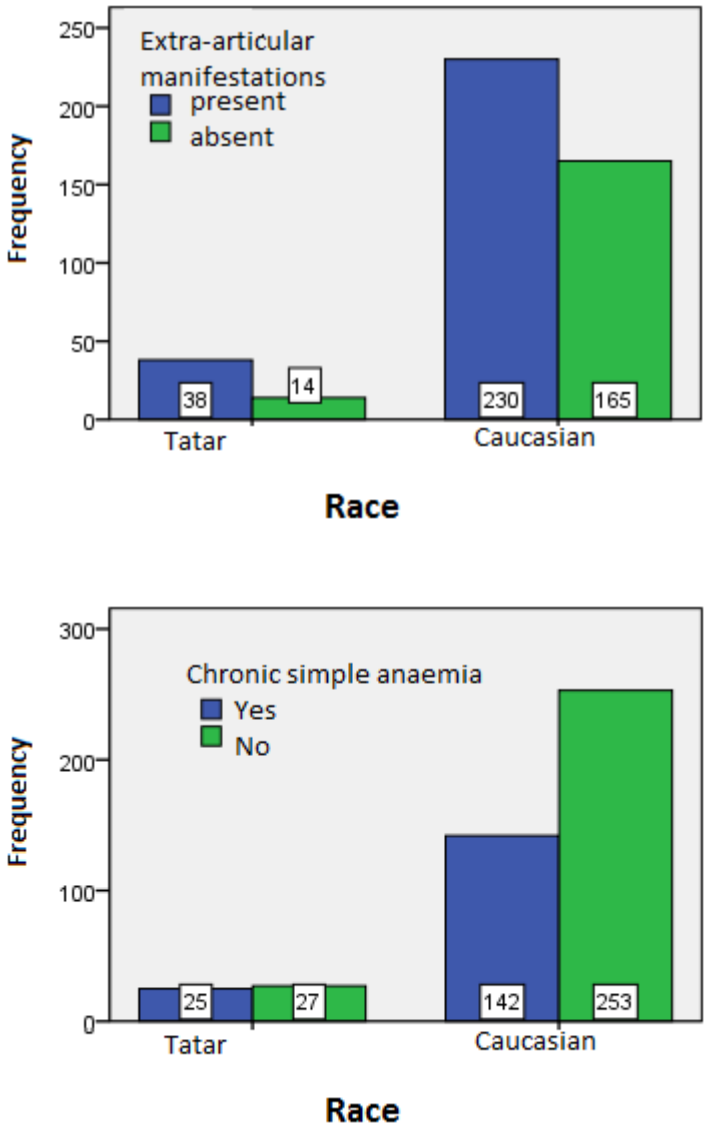

Figure 2. Extra-articular manifestations according to patient ethnicity

Tatar RA patients also exhibit more frequent subcutaneous rheumatoid nodules, pulmonary rheumatoid nodules and episcleritis, but differences regarding the frequency of these manifestations amongst patients from the two ethnic groups do not reach the statistical significance threshold. Anti-CCP antibody presence and titres are higher in Tatar patients, but statistically significant differences are registered only in what regards the mean value of these antibodies (Table 2).

Table 2. Clinical characteristics of Tatar RA patients

Disease characteristics	**Caucasians**	**Tatars**	**p**
No.	395	52	
Disease duration	10.58±8.6	12.0±10.2	ns
Early disease	47 (11.9%)	9 (17.3%)	ns
RF	320 (81%)	39 (75.0%)	ns
RF>3n	242 (75.6%)	32 (82.1%)	ns
Anti-CCP	242 (63.5%)	36 (69.2%)	ns
Anti-CCP >3n	242 (63.5%)	35 (67.3%)	Ns
Anti-CCP (mean and standard deviation mean)	172.76±179.18 (0.2-1374.00) Mean : 104. 00	271.4±796.1 (0.4-5773.0) Mean : 110.50	***0.023***
Erosions	295 (74.7%)	42 (80.8%)	Ns
Ankyloses	119 (30.1%)	20 (38.5%)	Ns
Extra-articular manifestations	230 (58.2%)	38 (73.8%)	***0.03*** ***OR=1.947***
Simple chronic anaemia	142 (35.9%)	25 (48.1%)	0.06
Rheumatoid nodules	96 (24.3%)	14 (26.9%)	Ns
Pleuropulmonary manifestations	38 (9.6)	7 (13.5%)	Ns
Pulmonary rheumatoid nodules	7 (1.8%)	3 (5.8%)	Ns
Pleurisy	6 (1.5%)	2 (3.8%)	Ns
Episcleritis	4 (1%)	1 (1.9%)	Ns

The degree of disease activity, regardless of the measurement tool used, does not yield significant differences between the two ethnic groups.

Disease or treatment-related complications analysed in our study were represented by arthroplasty, stress fractures (vertebral and nonvertebral fractures) and aseptic osteonecrosis (localised, in all cases, at the femoral head). All though fracture rates and aseptic osteonecrosis cases were frequent in Tatar patients, differences were small and did not reach the statistical significance threshold.

Obesity, diabetes mellitus, HTN and ischemic coronary disease are present in similar percentages in RA patients from both ethnic groups. However, it must be noted that Tatar RA patients are more frequently affected by dyslipidemia ($p<0.001$) and diabetes mellitus, while HTN is more commonly found in Caucasian patients. Serum cholesterol and triglyceride levels have significantly higher mean values in Tatar patients compared to Caucasians.

SCORE values are similar in both groups of patients and they can be multiplied by 1.5 in both groups, the conditions for increased cardiovascular risk being met.

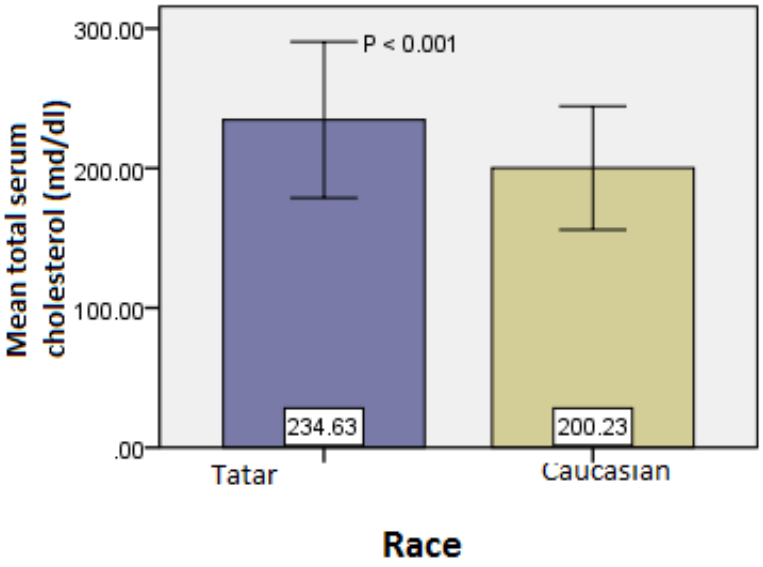

Figure 5. Serum cholesterol mean values in Tatar RA patients

Tatar RA patients also have an increased cardiovascular risk (SCORE>5%), similar to Caucasian patients. However, cardiovascular events are more common amongst Caucasian patients, possibly also due to an increased compliance of Tatar patients to statin treatment (Table 5).

Table 5. Comorbidities and cardiovascular risk factors in Tatar RA patients

Comorbidities	**Caucasians**	**Tatars**	**P**
No.	395	52	
BMI	27.26±5.17	26.6±5.2	Ns
Obesity	98 (25%)	12 (23%)	**Ns**
HTN	228 (57.7%)	24 (46.2%)	Ns
Dyslipidemia (CT > 200mg/dl) (no =213)	177 (45%) (239.10±30.68)	36 (70%) (259.66±45.15)	***0.001***
Total serum cholesterol	200.22±44.20	234.63±55.93	***> 0.001***
TG	130.17±50.98	148.96±54.87	***0.014***
Statin treatment	96 (24.3%)	19 (36.5%)	***0.045***
Diabetes mellitus	68 (17.3%)	12 (23.1%)	Ns
Disease duration	10.58±8.6	12.0±10.2	Ns
Extra-articular manifestations	230 (58.2%)	38 (73.8%)	***0.03*** ***OR=1.947***

Age mean	62.24±11.5	61.27±10.7	Ns
SCORE	7.28±9.96	7.13±8.42	Ns
SCORE > 5%	172 (43.5%)	24 (46.2%)	Ns
Ischemic coronary disease	63 (15.9%)	6 (11.5%)	Ns
Myocardial infarction	10 (2.5%)	0 (0%)	Ns
Cerebral stroke	21 (5.3%)	2 (3.8%)	Ns
Cancers	17 (4.3%)	2 (3.8%)	Ns
HBsAg (+)	14 (3.5%)	3 (5.8%)	Ns
Anti HVC (+)	29 (7.3%)	2 (3.8%)	Ns

Malignant neoplasia and chronic hepatitis B and C viral infections are similar in both ethnic groups.

VI. Discussions

DISCUSSIONS REGARDING THE INFLUENCE OF DEMOGRAPHIC FACTORS ON THE EPIDEMIOLOGY OF RHEUMTOID ARHTRITIS

The epidemiology of rheumatoid arthritis is characterised by a disease preference for women ages over 50, a classical female-to-male ratio of ≤ 3:1 and peak of disease incidence between 50 and 75 years old being described (1). As evidenced by the female-to-male ratio distribution, as well as by an increased risk in nulliparaous women, the association between pregnancy and remission, a decreased risk of rheumatoid arthritis in breastfeeding women, the relationship between female sex hormones and disease susceptibility is clear, but not yet completely understood (2).

Regional differences in disease prevalence support the theory of environmental factor influence on the development of rheumatoid arthritis. For the time being, with a few exceptions, such as smoking, the vast majority of risk factors remain unidentified, although their contribution to disease development is probably significant. The interaction between female sex hormones, genetic factors and smoking, as an environmental factor, is suggested by a series of clinical studies (3,4).

Given the alarming increase in the number of obese and overweight individuals during the last two decades (5), more and more studies outline the role of obesity in the

onset, evolution, and response to treatment of rheumatoid arthritis, although current data remains contradictory.

Latitude represent a geographic indicator used as a surrogate for the study of environmental influence, and of all of its components (geo-climatic conditions, industrialisation and pollution, socio-economic status and lifestyle) on the epidemiologic characteristics of rheumatoid arthritis in various geographic regions of the world. In Europe, for example, a north-south gradient is discussed – with an increased prevalence of rheumatoid arthritis in Northern countries compared to Southern countries (6). The American continent also seems to be characterized by a north-south gradient, which influences age at onset and gender distribution: rheumatoid arthritis has an approximately 12 years earlier onset in Mexican patients compared to Canadians, while the Mexican sample population is characterized by a percentage of 91% women, compared to the Canadian sample population, which registers a percentage of 77% women (7).

DISCUSSIONS REGARDING DISEASE CHARACTERISTICS

Our sample population is characterised by "real life" patients. Their clinical and demographic characteristics were compared, on most occasions, with those of patients also selected from real life those from the QUEST-RA clinical studies (The Quantitative Standard Monitoring of Patients with RA). These studies are part of a programme initiated in 2005 in order to encourage the quantitative evaluation of rheumatoid arthritis and to establish an international database for rheumatoid arthritis patients from several countries, with a standard monitoring either in a hospital or outpatient context. Within this programme, numerous other clinical studies, which set the goal to evaluate various demographic and clinical characteristics of rheumatoid arthritis patients in numerous European, North American, and South American countries, were initiated.

The demographic and clinical characteristics of our sample populations were also compared to those of the rheumatoid arthritis patients from the COMORA study. Conducted between 2011 and 2012 (approximately the same period as our study), published in 2014, COMORA is a transversal study that included 3920 adult rheumatoid arthritis patients from 17countries on 4 continents: Europe, Asia, North America, and

South America. The study's primary goal was to determine the prevalence of comorbidities and to evaluate their monitoring, but data regarding demographic and clinical characteristics of patients and disease were also collected and published (1).

Compared to the sample populations from the different countries included in the COMORA study, our sample population is characterized by a higher number of patients, a higher percentage of women, of obese and overweight patients, a median age over 60 years old, a DAS28 ESR value corresponding to the moderate disease activity interval, a higher rate of NSAIDs consumers, and a below average number of biologic agents prescriptions. The percentage of patients under current glucocorticosteroid treatment is lower than the sample population average, lower than in several European countries. From the demographic and clinical characteristics synoptic table, the most surprising element is represented by the average DAS28 ESR disease activity score, which is surpassed only by the Egypt and Morocco sample populations. This is surprising, as the patients monitored by out department are carefully evaluated according to "treat to target" monitoring, they benefit from regular medication adjustments, and biologic treatment initiations when the National Insurance Agency criteria are met.

DISCUSSIONS REGARDING TATAR RHEUMATOID ARTHRITIS PATIENTS IN CONSTANTA COUNTY

RA development risk varies according to ethnicity, as the disease is more frequent amongst Caucasian populations and registers lower prevalence rates in African and Asian countries. RA prevalence amongst the Mongoloid race is under 0.5% of the general population. At least in some of the Asian countries with Mongoloid populations, such as Japan, RA has registered a downward trend, especially amongst women, over the past decades.

In 1996, RA, as defined by the ACR 1987 criteria, registered a total prevalence of 0.2% (0.14% for men and 0.24 for women) in the Japanese province of Kamitonda, lower than that registered between 1969-1985 (0.47% in 1969 and 0.35% in 1985). Disease incidence decreased from 0.35 to 1000 people between 1965-1975 to 0.09 to 1000 people in the 1985-1996 decade (1). The decrease in disease incidence, which has been observed

globally in the last few decades, is correlated with the use of birth control medication; however, in Japan, the use of such medication is reduced (approximately 1% of sexually active females). The main modifications that have intervened in the lives of Japanese females are of a social nature: increase in employment rates, increase in economic independence status, increase in education levels, and a decrease in the number of pregnancies. These factors have lead to a stronger female involvement in social activities and responsibilities, and increase in social role, the statute of "female-male" being attributed to a higher concentration of androngens. According to James' theory, androgren hormones are the ones that prevent the development of RA (2).

Rheumatoid arthritis, as defined by the ACR criteria, registers a prevalence between 0.2-0.37% in continental China, without major differences between the Northern and Southern regions, rural or urban environments, or amongst different ethnic groups (3). In Taiwan, RA registers an increased prevalence of 0.93% in urban areas and a prevalence of 0.26% in rural areas, similar to that registered by continental China (3). In Taiwan, in urban and suburban areas, the higher prevalence rate (0.93%) is possibly linked with a superior development of this area compared to continental China, as socio-economic status is regarded as a potential factor in the development of the disease. The results of a epidemiologic study regarding rheumathologic diseases in China, published in 2012, estimate a RA prevalence of 0.28% in the general population, with a female-to-male ratio of 6:1, varying from 2:1 for the 36-45 years old decade to 14:1 for those over the age of 66. RA prevalence in China is similar to that of Asian states with Mongoloid populations (Vietnam, Indonesia, the Philippines), higher than in Japan, but lower than that in Asian countries with Caucasian population (0.55% in Pakistan for people over the age of 15) (4).

Ethnic difference in RA prevalence is largely linked with genetic factors, but the influence of environmental factors must also be taken into account. The most frequent HLA alleles associated with RA amongst the Caucasian population are HLA-DRB1*0401 and HLA-DRB1*0404 (5), while the HLA-DRB1*0405 allele is most frequently observed in RA patients in East Asia (6). The HLA-DRB1*0901 allele is also more frequently observed in Asian RA patients compared to European patients.

Consequently, amongst the Korean population, heterozygotes for the HLA DRB1 0405 or 0901 alleles have an up to 60 times higher risk for developing RA (6).

Constanta County is home to over 80% of Romania's entire Tatar population. Previous clinical observations of our peers have suggested a higher prevalence and a higher degree of severity amongst the county's Tatar population. The results of our study support this clinical observation, even though it was not initially intended as an evaluation study of the disease prevalence amongst the two populations. According to the last census, the Tatar population in Constanta County represents 3.3% of the total population; in our sample population, the percentage of Tatar patients amounted to 11.6%. With the use of statistical analysis, the percentage of Tatar RA patients was compared to the entire Tatar population of the county, and the percentage of Caucasian RA patients was compared to the entire Caucasian population: 0.21% and 0.05% respectively. These numbers clearly show a higher percentage of RA cases amongst the Tatar population, but they cannot estimate the prevalence of RA in Constanta County, even though the Clinical Emergency County Hospital "Sf. Apostol Andrei' department of rheumatology is the most important in the south-eastern region of the country. In our county, three other rheumatology departments and at least three more private practices that service the population exist.

No published studies regarding RA epidemiology exist in Romania; thus, the estimation of a 1% prevalence based on the geographic conditions of our country and the preponderantly Caucasian population is very likely unrealistic (7). Moreover, in 2012, Romania registered approximately 40.000 prescriptions for disease modifying agents, which would amount to a global prevalence of 0.2% (8). No data regarding the prevalence of RA in Tatar populations scattered across certain territories of the Russian Democratic Federative republic, in the union states of the Commonwealth of the Independent States, in Crimea and in Turkey (registered as Crimean Turks).

No revealing studies regarding genotypes of RA patients exist in Romania and neither do any Tatar communities across the globe benefit from such genetic studies. Moreover, no comparative analyses of non-genetic risk factors (nutritional factors, autoantibodies, obesity, smoking, and so on) amongst two different ethnic populations which share the same geographical and socioeconomic conditions, but register different

disease prevalence rates exist. The only information regarding the genetic profile of Caucasian and Tatar RA patients are derived from the results of a doctoral study, also conducted in our department, a study which set out to analyse the association between phenotype and genotype in cases of early RA. In the Caucasian group of RA patients, the highest relative risk was calculated for the * 10 allele (RR=4.63), followed by the *04 allele (RA=2.07), while the lowest rates were registered for the *01 and *14 alleles with a RR of 1.91 and 1.81 respectively. A distinct, but unfortunately insufficient in what regards number of patients, genotype was represented by Asian patients, who were primarily characterized by the *04 allele, closely followed by *07 and *15, and less by the *01, *03, and *14 alleles (9). This relative rare set of DRB1*10 alleles, present in Caucasian RA patients from the sample population studied by Pazara et al, which was associated with the highest relative risk and the most statistically significant frequency, was also observed in Portuguese populations, but also in other Southern European countries (10).

In our sample population, Tatar RA patients are characterized by a younger age at onset, an increased number of extra-articular manifestations (especially chronic simple anaemia, pleurisy, and rheumatoid pulmonary nodules) and by high antiCCP titres.

The prevalence of RA in Tatars seems to be higher than that amongst the Caucasian population in Constanta County and the data we have obtained is according to recent reports regarding the prevalence of this disease in Asian populations. Consequently, the notion of decreased prevalence in Mongoloid populations seems to be obsolete: new data regarding RA prevalence in Japan, based on the medical reports of the Japan Medical Data Center, estimates a disease prevalence between 0.6-1%, numbers that are significantly higher than earlier reports according to which Japan was amongst the countries with a low prevalence of rheumatoid arthritis (12). Further observational epidemiological studies are required in order to confirm this data. Our goal is to continue the research of Caucasia and Tatar patient genotypes and the potential epigenetic alterations induced by environmental factors.

VII. Conclusions

1. Our sample population is characterized by a mean age in the 7th decade of life, a possible consequence of the process of demographic ageing Romania is experiencing (a decrease in birth rate, an increase in life expectancy, migration towards other European countries of the active population).

2. The F:M ratio in our study (6:1) is almost double than the one described in literature and is similar to that registered by countries with a precarious socio-economic status, regardless of their geographical localization or climate.

3. The F:M ratio registers important changes according to current age and age at onset, based on the smoking status at disease onset or in the period before the onset, according to area of residence and body mass index – early age onset, non-smoker status, residence in the rural continental area of the county and obesity increase the F:M ratio among the study population.

4. Our study outlines a particular demographic tendency for RA patients over the past 5 years, characterized by an increase in age at onset for both sexes.

5. A downward trend for the incidence of RA cases is not noted: between 2003 and 2013 over 20 new cases of RA were registered yearly (between 20 and 30 cases, with a mean of 24). However, the number of RA cases in women has decreased, with a F:M ratio of 3:1 for the past 3 years. The change in gender distribution can be a part of the downward trend for the incidence of rheumatoid arthritis in women in particular.

6. The diagnosis was established in the first three months in 34% of patients, in the first 6 months in 52.1% and in the first year in 74.3% of the patients in our study. The interval between onset and diagnosis is significantly lower for cases with a disease duration of ≤ 3 years: 6.53±7.18 months (a mean of 3 months) as opposed to 18.75±26.44 months (a mean of 9 months), which represents the interval between onset and diagnosis for cases with a disease duration higher than 3 years.

7. Smoking influences age at onset differently: smoking leads to a significant increase in age at onset for the male with early onset disease (≤ 52 years old) and lowers age at onset for both sexes, the differences being significant only in the case of females with rheumatoid arthritis and late onset (>52 years old). Onset at an early age is genetically conditioned.

8. RA leads to invalidity and functional handicap: in the first ten years of illness, 47% of patients retire from ill-health.

9. Rheumatoid factor (RF) and/or anti CCP antibodies are present in 82.1% of the patients in our study, which places our sample population amongst those with a high percentage of seropositivity.

10. The rheumatoid factor is associated with anti CCP antibodies in 81.3% of patients, while cases with positive anti CCP bodies are associated with the RF in 97.3%. The rate of cases with negative RF and positive anti CCP antibodies is very small, under 1%.

11. The presence of the rheumatoid factor or anti CCP antibodies is significantly correlated with onset at a young age and with a series of clinical and biological characteristics of the disease (the presence of erosions and ankyloses, the presence of extra-articular manifestations – especially chronic simple anaemia and rheumatoid nodules). These characteristics seem to be determined by the presence of the anti CCP antibodies and not that of the rheumatoid factor.

12. The rheumatoid factor, but not the anti CCP antibodies, is correlated with disease activity.

13. Erosions appear early on: in 19.6% of cases they are present in the first year of illness and in 27.4% of cases in the first three years of illness. For patients with erosive disease, a increase in the onset-diagnosis interval is registered, the delay in diagnosis being associated with an increase number of patients with erosions.

14. Acute phase reactants (CRP, ESR) have according values in only half of the analysed cases.

15. The mean ESR value is significantly correlated with all composite scores ($p<0.001$) and with each element of these scores (P-VAS, TJC, SJC) ($p<0.001$), with the exception of the C-reactive protein ($p=0.455$).

16. There are no significant associations between the mean CRP value and the other elements of the composite scores: P-VAS, TJC, SJC, ESR. The mean CRP value is associated with the degrees of disease activity only in cases in which is measured through the DAS28 CRP score ($p<0.001$) and it approaches the statistical significance limit in case of evaluation through the SDAI score ($p=0.055$).

17. The degree of disease activity is appreciated differently according to the evaluation score used.

18. The limit between remission and low activity is fragile and conditioned primarily by purely subjective components of the evaluation tools. In our study, the classification of patients as being in remission or having low activity disease is exclusively conditioned by subjective evaluation components – P-VAS, E-VAS, TJC – regardless of the composite score used.

19. There are important discrepancies between the subjective and the objective components of the evaluation scores, which urge the need to increase objective methods for assessment of inflammation degree.

20. Early disease, the presence of extra-articular manifestation and of high RF titres are correlated with increased disease activity.

21. The most frequent extra-articular manifestation present in our sample population were chronic simple anaemia and subcutaneous rheumatoid nodules.

22. Severe cardiovascular events were conditioned by traditional risk factors. No correlations between them and disease duration, the presence of extra-articular manifestations, of the rheumatoid factor or anti CCP antibodies were noted.

23. The results obtained in our study support the hypothesis of an increased prevalence of the disease amongst the Tatar population.

24. Tatar RA patients are characterized by a younger age at onset, a increased number of extra-articular manifestations (especially simple chronic anaemia, pleurisy, and rheumatoid pulmonary nodules) and high antiCCP titres.

25. Significant differences can be observed between the demographic characteristics of patients belonging to the two ethnicities in what regards the socio-economic aspect. Tatar patients register more ill-health retirements (possibly due to younger age at onset and longer disease duration) and, consequently, a higher number of patients on welfare.

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