UNIVERSITY „OVIDIUS" CONSTANŢA
DOCTORAL SCHOOL
DOCTORAL TRAINING PROGRAMME - MEDICINE

Influence of smoking on the appearence and evolution of rheumatoid arthritis

- Phd Thesis Summary-

PhD Scientific Coordinator:
Prof.Univ.Dr. Maria Şuţa

PhD Candidate:
Daniela Gherghe (Duşa)

CONSTANŢA
2018

TABLE OF CONTENTS

Key words: rheumatoid arthritis, smoking, incidence, seropositivity, erosions, extraarticular manifestations, response to treatment

Note: The tables and figures inserted in the abstract of the doctoral thesis retain the original numbering in the thesis. The content of the abstract is the one found in the PhD thesis.

Introduction

Rheumatoid arthritis (RA) is a chronic immune-mediated inflamatory disorder with predominantly articular manifestations, but also with multiple systemic manifestations. RA accounts for approximately 10% of all rheumatic diseases, with an annual incidence of 30/100000 (1). The prevalence of RA is approximately 1% (2) and an increase of 22% between 2005 and 2025 is estimated due to the aging population (3).

Affecting especially the young people (30-55 years old), poor prognostic due to chronic and disabling evolution, with important systemic injury, have led reumatologists to develop diagnostic and classification criteria with increase sensitivity and specifity to allow early disease modifyind treatment, especially biological therapy. Although in the past years anti-rheumatic therapy had a major evolution, with the discovery of new molecules, RA is further associated with long-term morbidity and early mortality (3).

Efficient use of the new therapy did not lead to remission in all patients, leading to disability, deacresed quality of life and low work capacity. Initiation the treatment in the first year of illness has led to protection against radiological progression and disability (3).

Recent research defining a preclinical stage of the RA and have provided additional information on mechanisms that do the transition from health to disease states. This informations have founded a prevention stategy of RA, which is a major and a significant change of the therapeutic behavior (3).

Another important step in RA prevention took place in October 2016, when EULAR defined the characteristics of arthralgia suspicious for progression to RA (4).

Primary prevention methods consist of life style change and the knowledge of etiopathogenic factors become priority. Genetics, autoimmunity, infectious agents, oral contraceptives, smoking, education level were incriminated in the appearance of RA.

Smoking is the largest environment risk factor involved in the etiology of RA (5). This association was described 25 years ago, but could have been explained over the past 10 years with the discovery of the role of anti-CCP antibodies.

Knowing the mechanisms by which smoking causes RA and the way in which these environmental factors influence the evolution of RA are the subjects of this thesis and can be premises to create local and

national programs for prevention of RA. Is important to inform the population about the role of smoking on the development ad evolution of RA, and the smoking cessation is recommended to patients with RA.

Part of the results were presented in national scientific events, with abstracts published in the volumes of abstracts: National Congress of Rheumatology (2014 – " Rheumatoid Arthritis and Smoking - Epidemiological Study in the County of Constanța" - e-poster, 2015 – "Smoking - an unfavorable prognostic factor in patients with rheumatoid arthritis?" - oral communication, 2018 – "Smoking and rheumatoid arthritis retrospective study conducted in Constanţa County" - oral communication), National Conference on Tabacology (2015 – "The Impact of Smoking on the Rheumatoid Arthritis severity" - oral communication), National Congress of the Romanian Society of Pathophysiology (2015 – "Influence of immunological aspects on the evolution of rheumatid arthritis" – poster, 2017 – "Can smoking influence the evolution of rheumatoid arthritis?" – poster), National Conference of the Society of Physiology (2015 – " Rheumatoid arthritis in smokers – a more active disease?" – poster) and the "Dobrogene Medical Days - 25 Years of Medical Education" Conference (2015 - "Smoking influences the evolution of rheumatoid arthritis" - oral communication). In April 2018 some results of the study were presented at the *XVII* Mediterranean Congress of Rheumatology in Genoa, Italy as a poster titled "Smoking and rheumatoid arthritis" and the abstract was published in *Clinical and Experimental Rheumatology* .

The doctoral thesis is structured according to the classical model, and comprises a general part made up of four chapters with a total of 34 pages, and a personal part, a personal research, consisting of six chapters of 82 pages, 290 bibliographical reference, 47 figures, 25 tables and 29 annexes.

General Part

Chapter 1 addresses epidemiology of rheumatoid arthritis .

Chapter 2 is reserved to the aetiology of rheumatoid arthritis and is divided into subchapters analyzing issues related to genetics, predisposing factors, and of these, in particular, sex, factors related to reproduction, infectious agents, autoimmunity and environmental factors.

Chapter 3 of the general section analyzes the effects of smoking on inflammation and immunity. We have tracked the effects it has on circulating inflammatory cells (leukocytosis due to the increase in neutrophil counts, with changes of there phenotypic), on the mediators from peripheral blood (acute phase proteins and cytokines), on the macrophages (decrease of the phagocytic function, inhibition of IL-1, IFN gamma and TNF alpha) and on the lymphocytes (the energy of T lymphocytes). Three subchapters described in detail the connection between smoking, the production of anti-CCP antibodies in the lungs, the presence of the common epitope and the appearance of rheumatoid arthritis.

Chapter 4 is an analysis of the known and demonstrated aspects so far about the relationship between smoking and rheumatoid arthritis.

Personal Study includes personal research, the aim of the thesis, the research obsectives, the material and the method. A final chapter is dedicated to the results and conclusions.

The propuse of this research is to archieve in our county the first study that has as main objective the examination of the effects of smoking on rheumatoid arthritis, trying to follow all aspects of the disease which are influenced by the incriminated environmental factor (pathogenesis, clinical manifestations, laboratory tests, extraarticulare manifestations, disease activity, complications, prognosis, treatment).

The main objective

The study aims to carry out a detailed amnalysis of how exposure to smokig influences the appearance and evolution of rheumatoid arthritis in patients in Constanţa Country hospitalized in the Emergency Country Hospital “Saint Andrew” Constanţa.

Secondary goals

1. Description of the patient with RA, referring to the socio-demographic characteristics (age, sex, smoking status), the history and characteristics of the disease (onset age, disease, seropositivity, stage, disease activity, extraarticular manifestations, treatment)
2. Highlighting the clinical, biological and functional features of rheumatoid arthritis is the two populations groups (smoking / non-smoking)

3. Establishing correlations between smoking and the occurrence of rheumatoid arthritis (especially the age of onset of the disease)

4. Establishing correlations between smoking and clinical manifestations of rheumatoid arthritis (seropositivity, activity, extraarticular mannifestations)

5. Establishing correlations between smoking and treatment response (number of DMARDs', inefficiency)

Material and method of study

In order to achieve the proposed objectives, we performed an analytical clinical study (case / cohort) in which we included 28 5 patients with rheumatoid arthritis (according to ACR 1987 and EULAR 2010 criteria) admitted to the Department of Rheumatology of the Clinical Medical Clinic II County Emergency "Saint Andrew" Constanţa.

Patients were evaluated during continuous admission in the department or in day hospitalization by a rheumatologist from our department. Patients agreed to participate in the study by signing and dating an informed consent.

Selection of patients

Inclusion criteria:

1. Certain diagnosis of rheumatoid arthritis according to ACR 1987 and / or ACR / EULAR 2010
2. Age> 18years

Exclusion criteria:

1. Patients with early arthritis that can not yet be diagnosed as rheumatoid arthritis
2. The lack of medical records revealing information about the history and characteristics of rheumatoid arthritis

Patient evaluation comprised:

- Clinical evaluation of the patients: socio-demographic characteristics (age, sex) and the history and feature of RA (age on the onset, duration of the disease, radiological staging, extraarticular manifestation, disease activity)
- Paraclinic evaluation of patients: biological inflammation syndrome (ESR, CRP), autoantibodies specific to rheumatoid arthritis (RF, antiCCP antibodies).
- Smoking assessment: placing patients in one of the categories - smoking / non-smoking at the onset of the disease, smoking / no smoking at the time of evaluation, smokers / never smokers -, quantification of tobacco consumption by pack years.

Results

The characteristics of the patients of the studied group

The socio-economic characteristics of patients with Rheumatoid arthritis who have met the criteria for inclusion in the study are presented in Table XII.

Table XII. Socio-economic characteristics of the patients

Socio-economic characteristics	n= 285 patients
Age, mean± SD(years)	62,02 ± 11,72
Women (no., %)	244 (85,6)
Men(no., %)	41 (14,4)
Women:Men Rate	6:1
Urban area (no., %)	229 (80,4)
Rural area (no., %)	56 (19,6)
Urban : Rural rate	4:1
Caucasians (no., %)	258 (90,5)

Clinical characteristics of RA patients

Tabel XV. Clinical characteristics of RA patients

Clinical characteristics	n= 285 patients
Age, mean on onset of disease ± SD (years)	51,15±14,018
Age of disease ± SD (ani)	11,28±9,72
Early form (no., %)	30 (10,5)
Etablished form (no., %)	255 (89,5)
RF present (no., %)	231 (81,0)
Ac anti CCP present (no., %)	173 (70,3)
Presence of erosion (no., %)	218 (76,5)
Presence of ankyloses (no., %)	98 (34,4)

The extraarticular manifestations were diagnosed in 194 patients (68.1%), and the highest incidence of these had chronic anemia (158 patients, 55.4%), followed by rheumatoid nodules 71 patients, 24.9%), vasculitis (16 patients, 5.6%), pulmonary fibrosis (15 patients, 5.3%). Four patients (1.4%) associated carpal canal syndrome, respectively serotypes.

Evaluation of the activity of rheumatoid arthritis (Table XVIII) involves the evaluation of the joint symptoms represented by the degree of objective and subjective affection of small and large join.

Tabel XVIII. Values of the main indicators of disease activity

	N	Minimum	Maximum	Average	Standard deviation
DAS28ESR	285	1,40	9,12	4,5561	1,6895
DAS28CRP	285	0,96	7,62	3,5176	1,5971
CDAI	285	1	75	19,1175	15,0055
SDAI	285	1	87	20,7766	16,494
EGA	285	10	100	41,26	25,7524
VAS	285	0	100	48,92	25,739
TJC	285	0	28	7,57	7,6668
SJC	285	0	28	2,57	4,286
ESR	285	2	120	36,2	24,217
CRP	285	0,00	18,.00	1,6478	2,647
Valid N	285				

Based on the statistical analysis of the correlations between the 4 composite indices expressing the activity of rheumatoid arthritis, $p < 0.001$ with statistical significance was obtained in all cases, DAS_{28} VSH correlated strongly with both DAS_{28} PCR, CDAI and SDAI ($r = 0.922$, $r = 0.895$ and $r = 0.897$ respectively).

By the anamnesis we found out that there were patients that were diagnosed with rheumatoid arthritis in the first month of the onset of symptoms. There is a patients which was diagnosed 22 years after the begining of joints pain. In 75% of patients the diagnosis was estableshed in the first year after the onset of the arthritis. Average time from the onset of symptoms to the diagnosis of rheumatoid arthritis was 16,45 ± 28,87 months.

This time is important because in this way we can determine how early the disease modifying treatment was initiated. Regarding the first DMARs, 57% of patients started with Methotrexate, the first line drug in rheumatoid arthritis (Figure 21). The patients who have started thetreatment with gold salts are patients diagnosed in the 80s. Th first medicine was another disease-modifying therapies may be due to lack of Methotrexate on romainian market or because of the contraindications of Methotrexate.

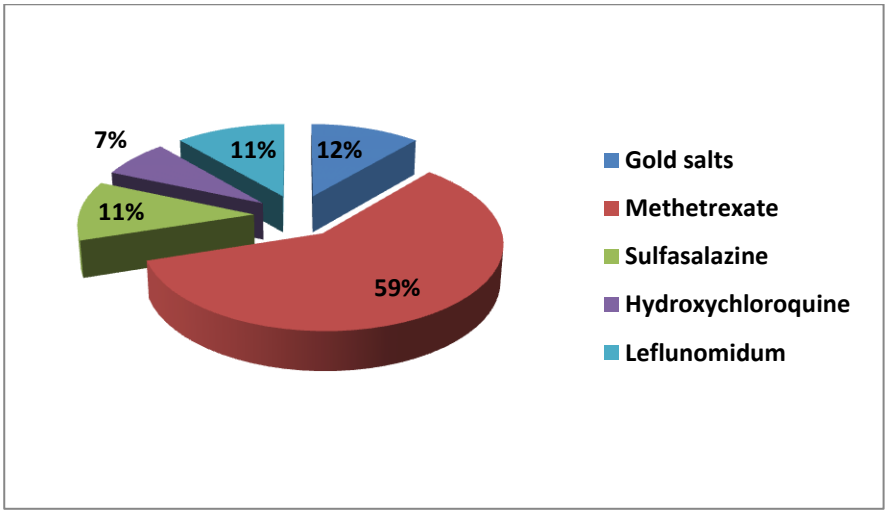

Figure 21. Initial treatment of patients in the study group

Currently, the entire batch of patients is in treatment with one or more disease modifying drugs. A total of 78 patients (27.37%) received biological therapy, 37 of whom were in treatment with an anti-TNF-alpha agent and 41 with an anti-CD 20 agent (Figure 22). According to national treatment protocols, biological therapy is associated with a conventional DMARD (monotherapy or combination therapy). In our study, most patients were receiving combination therapy (74 patients – 94,87%), the rest were receiving monotherepy (3 patients with anti TNF alpha agent and a patient with anti CD 20 agent).

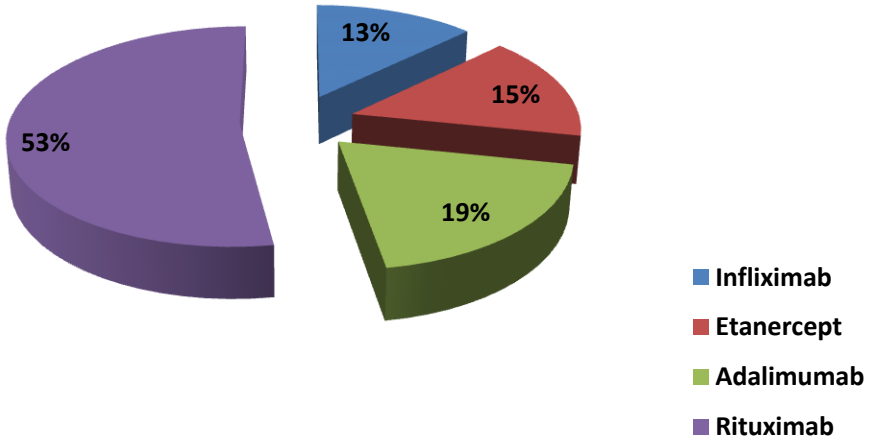

Figure 22. The biological therapy used by the patients in the studied group

Over half (51.57%) of patients had Methotrexate in their medication, whether monotherapy or combination therapy with a conventional and/or biological DMARDs. The percentage of patients treated with Sulfasalazine, Hydroxychloroquine and Leflunomidum in monotherapy or combination therapy with another conventional and/or biological DMARD is approximately the same (SZZ -27.01%, HCQ-29.12%, LEF-28.42%) (Table XX).

Table XX. The medication of the patients in the studied group

	MTX	SSZ	HCQ	LEF
Monotherapy	59	14	15	28
Monotherapy + anti-TNF alfa	11	1	1	7
Monotherapy + RTX	9	1	2	15
Combination therapy with DMARD	47	43	48	25
Combination therapy + anti-TNF alfa	12	9	9	3
Combination therapy + RTX	9	9	8	3
Total	**147**	**77**	**83**	**81**

Rheumatoid arthritis and smoking

Smoking in patients with rheumatoid arthritis

We noticed the smoking habits in the patients at the beginning of the disease and at the time of evaluation . The results are recorded in Table XXI.

Tablel XXI. The percentage of smokers in studied group

Smokers on the onset	71 (24,91%)
Current Smokers	34 (11,93%)
Ever smokers	96 (33,68%)

There are 5 categories of patients:

- patients who have never smoked 189 (66.31%)
- patients who smoked at the onset of the disease and continue to smoke at present 30 (10.52%)
- patients who smoked at the onset of the disease and who subsequently quit smoking 41 (14.38%)
- patients who did not smoke at the onset of the disease and who then started to smoke 4 (1.40%)

- did not smoke at the onset of smoking, smoked along the way, but no longer smokes 21 (7.36 %)

Smoker status in patients with rheumatoid arthritis correlated with male gender; the highest percentage was represented by ever smokers' men (78%), and the smallest in the femele ever smoker category (9%). According to the data analyzed, the strongest correlation is between male and ever smoker status (p <0.0001, OR = 1,000; 95% CI = 0.453-0.2209).

Regarding the age of smokers, whether women or men, the same age distribution is maintained, maintaining the same tendency to group most patients in the 55-65 year range (Figure 25).

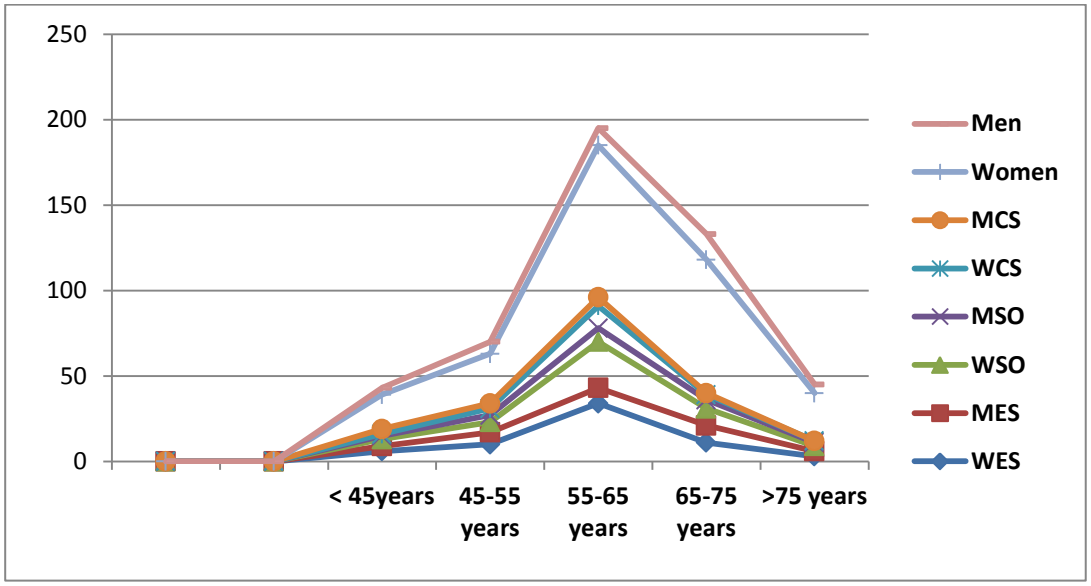

Figure 25. Distribution of patients in our group according the smoker status, age and sex

Mean age within the subgroup of never smokers was 63.42 ± 12.29 years compared with the subgroup of ever smoker patients, where the average was 59.27 ± 9.99ani, statistically significant difference (F = 8.178, p = 0.005), thus data indicating a lower age of ever smoking patients.

The average packs/year (PA) consumed by smokers on the onset was 17.17±12.34 and 81% of smokers on the onset were heavy smokers (average PA = 32.57 ± 12.47) compared with only 20% of the smokers on the onset (mean PA = 10.71 ± 18.66) (Table XXII).

There is also a direct linear relationship of low intensity between the age on the onset and the number of packs per year smokedon the onset of RA (r = 0.319, p = 0.007), but not between the age of the disease and the amount of tobacco consumed at the onset (r = 0.182, p = 0.128).

Table XXII. Tobacco consumption in smokers in the studied group

	N	Minimum	Maximum	Average	Standard deviation
Smokers on the onset	71	1	90	17.17	12.31
Current smokers	34	2	50	16.97	16.88
Ever smokers	96	1	90	17.74	21.24

According to the classification of the Romanian Society of Pneumology, we considered heavy smokers, smokers with a consumption greater than or equal to 20 PA. Of the total number of smokers, 38% (27 patients) of smokers on the onset and 38% (36 patients) of ever smokers were enrolled in the heavy smokers category. The percentage of heavy smokers enrolled is higher (41% - 14 patients) among current smokers (Figure 27).

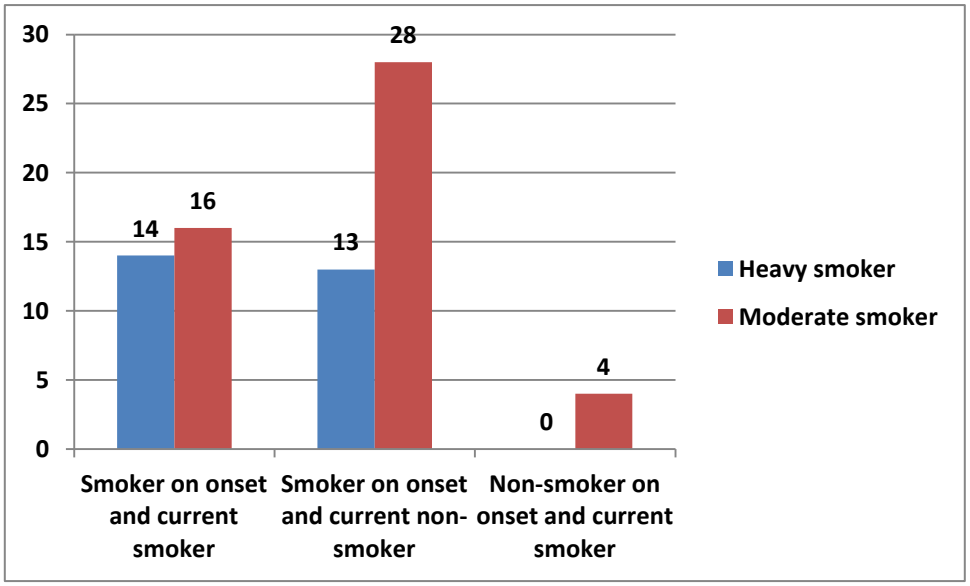

Figure 27. Distibution of smokers according to the amount of tabacco consumed

The percentage of heavy smokers is higher in the category of smokers at the onset and continue to smoke even now (47%). Among the patients who have started smoking after the onset of rheumatoid arthritis they are not heavy smokers.

Men from our studied group are smokers much heavier than women. 75% of male ever smoker smoke over 20PA, and among women only 19%. The highest percentage of smokers are among the male smoker at the onset of rheumatoid arthritis (81%). Heavy smoking status was statistically correlate with

male gender on the ever smokers (p <0.0001, OR = 0.0769, 95% CI = 0.0278-0.2127), the smokers on the onset (p <0.0001, OR = 0.0588, 95% CI = 0.0162-0.2139) and current smokers (p = 0.0056, OR = 0.098; 95% CI = 0.0819-0.5073).

Based on the statistical analysis of the average number of PA consumed in women and men, were obtained data showing that males with rheumatoid arthritis are heavy smokers than women both at the onset of the disease (F = 37,644; p <0,0010, or ever (F = 36,186; p <0,001).

Among the rural population at the onset of the disease were most smokers (72%), this is also the only correlation we found between the patients' home environment and the amount of tobacco consumed (p = 0.0199, OR = 0.1829, 95% CI = 0.0438-0.7648). Patients in rural areas smoke on the onset 26.4 ± 8.35 PA, with limits between 2 and 90PA, statistically significant (F = 12.647; p = 0.001) than patients in the urban environment (14.5 ± 13 32, with limits between 1 and 65).

The onset of rheumatoid arthritis and smoking

Analyzing the age of onset of rheumatoid arthritis in smokers and non-smokers, we could see that smokers develop illness earlier than about five years than non-smokers (Table XXIII). This difference is even greater when comparing the group of current smokers with current non-smokers (6.31 years).

Table XXIII. The mean age of onset of rheumatoid arthritis and smoking status

Ever smoker	48,13±14,01 years	
Never smoker	52,69±13,78 years	4,56 years
Smoker on the onset	48,72±11,84 years	
Non-smoker on the onset	51,96±14,58 years	3,24 years
Current smoker	45,59±13,76 years	
Current non-smoke	51,90±13,89 years	6,31 years

According to the ANOVA variation analysis, ever smokers have a significantly lower onset age than never smokers (F=6,902; p=0,009), as are current smokers versus current non-smokers (F=6,205; p=0,013).

The difference is even greater if we analyze the age at onset of the sub-groups divided by gender. Ever smoker female developed rheumatoid arthritis 8 years earlier than non-smokers female and smokers on the onset female 5 years earlier than non-smokers on the onset female. In these cases, statistically

significant values of ANOVA correlation indices were obtained (F = 5.428; p = 0.023 for smokers on the onset female and F = 4.985; p = 0.027 for ever smokers female).

We thought the analysis of the influence current smoking status on the age at the onset of rheumatoid arthritis it wasn't relevant, because as we mentioned above we have seen the dynamics of smoking habits, with a group of patients who did not smoke at the onset of the disease and who have started smoking during the course of the disease.

Men make illness later than women, and smoking seems to be of no particular influence, with the difference being 1-2 years between smokers and smokers, much less than women (Figure 30).

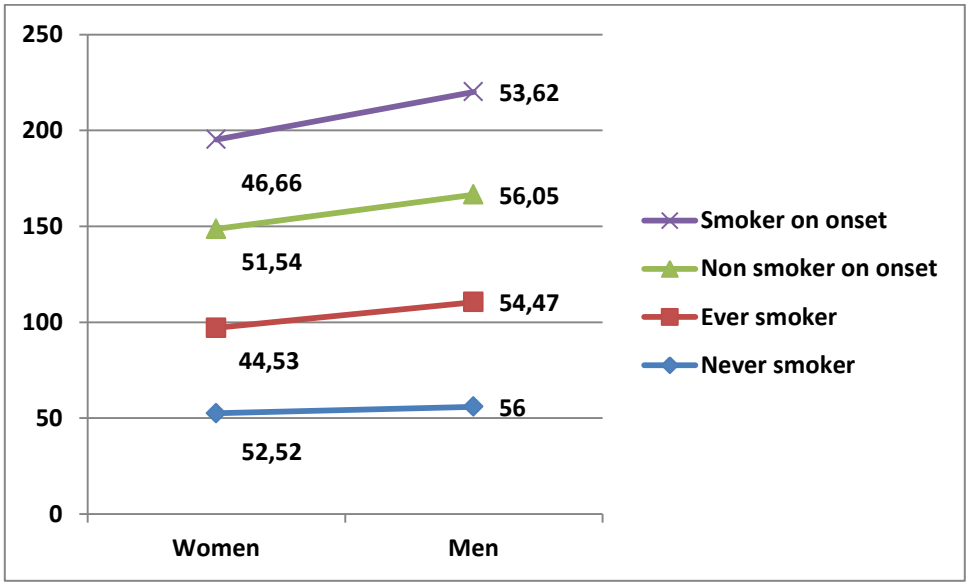

Figure 30. Age on the onset of rheumatoid arthritis accordind gender and smoking status

Smoking and seropositivity

Patients who smoke at the onset of the disease are seropositive for rheumatoid factor (90%) and antiCCP (80%), and non-smokers are seropositive 79% and 67%, respectively. From the point of view statistically, the presence of autoantibodies correlated with the smoking status at the onset of the disease (Figure 31).

In divided subgroups according to sex and presence of autoantibodies, the onset of smoking status correlated statistically significantly with male gender (p = 0.048842, chi-square statistic = 3.8807 for RF and p = 0.043614, chi-square statistically = 4.0714 for antiCCP antibodies). In fact, 90% of male smokers had rheumatoid factor, and 79% had antiCCPs compared to non-smokers where the percentages were 65% and 47%.

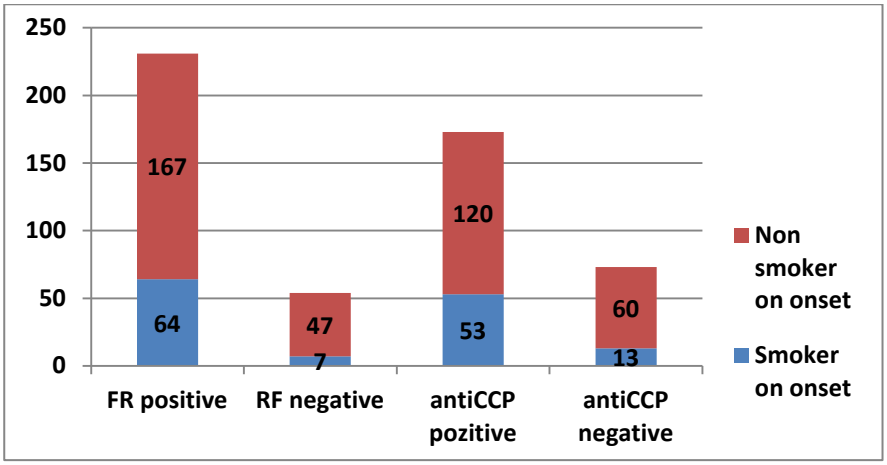

Figure 31. Correlations between the presence of autoantibodies and smoker status on the onset

Autoantibodies were present in higher percentages in Caucasian smokers, 89% rheumatoid factor in smokers versus 80% in non-smokers and anti-CCP 82% versus 68%. A statistically significant correlation was found between the anti-CCP positive and caucasian smoking status at the onset of the disease (p = 0.0359, OR = 2.1886, 95% CI = 1.0528-4.5496).

Rheumatoid factor was present in a higher proportion (95%) in smokers at the onset under 20PA compared to heavy smokers where the percentage is 81%. Calculation of the statistical chi-square test resulted in a value of 3,676, with a p = 0,0552, insignificantly statistically. It was not possible to show an association between the PA consumed at the onset of the disease and the rheumatoid factor value.

Smoking and the evolution of rheumatoid arthritis

Erosions were present in 77% of patients in the study group. Among smokers, this proportion remains, increasing to 85% for current smokers. In the case of non-smokers, the erosion rate remains around 75% (Figure 32).

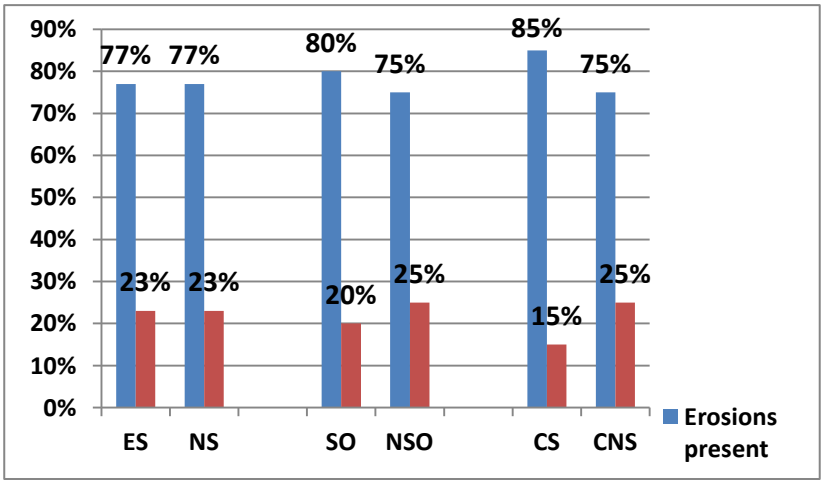

Figure 32. Correlations between the presence of erosions and smoker status

The percentage of extraarticular manifestations is higher for non-smokers with rheumatoid arthritis for each category of smokers, but this difference becomes statistically significant in current smokers who develop extraarticular manifestations at a frequency of 59% compared to current non-smokers develop extraarticular manifestations with a frequency of 70% (p = 0.0471, OR = 0.4794, 95% CI = 0.2320-0.9950).

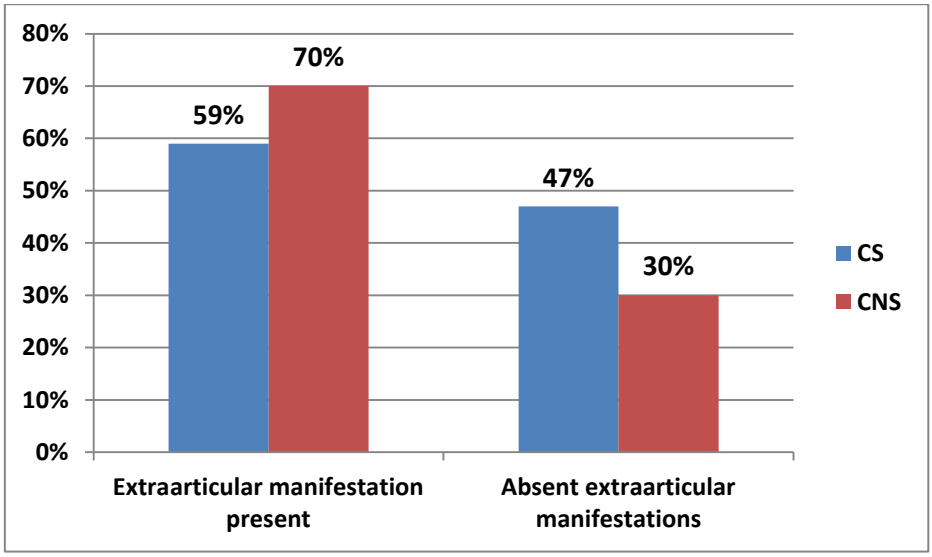

Figure 35. Correlations between the presence of extraarticular manifestations and current smoker

Out of the extraarticular manifestations we have watched the influence of smoking on rheumatoid nodules and anemia because they are the most common.

The status of the ever smoker and smoker at the onset of rheumatoid arthritis was associated with the presence of rheumatoid nodules (p = 0.0018, OR = 2.4486, 95% CI = 1.3958-4.2953, respectively p = 0.0004, OR = 2.9537; 95% CI = 1.6270 to 5.3622). Of the ever smokers, 40% had rheumatoid nodules, and also 45% of the current smokers. Among current non-smokers and never smokers, the proportion of rheumatoid nodules is 21%. Current smoker status is not correlated with the presence of rheumatoid nodules (p = 0.7146, OR = 1.1682; 95% CI = 0.5077-2.6880) (Figure 37).

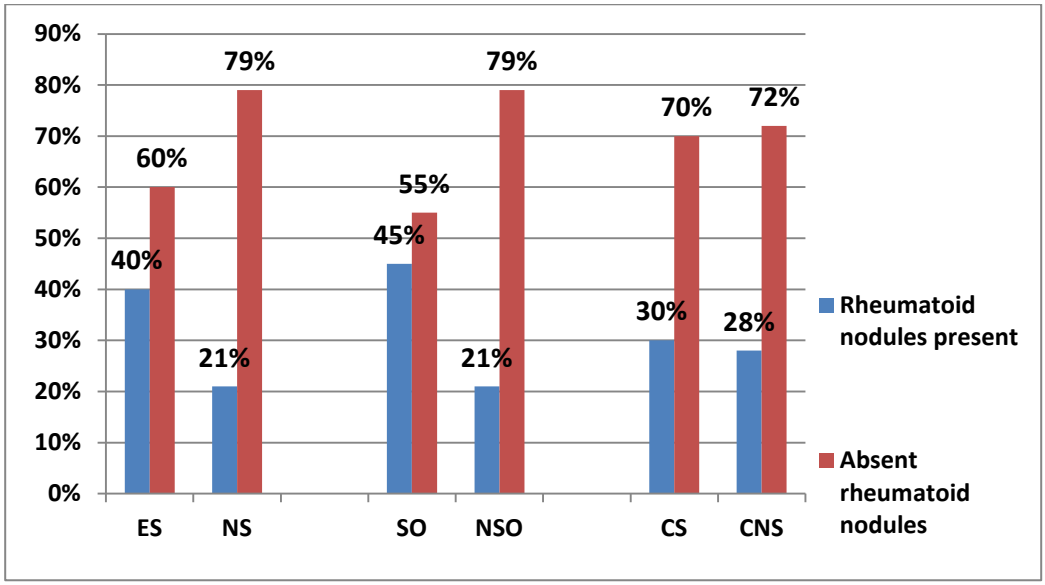

Figure 37. Correlations between smoker status and presence of rheumatoid nodules in the studied patients

Correlation is maintained in female ever smokers, 43% having rheumatoid nodules at least once in the course of rheumatoid arthritis (p = 0.0020, OR = 2.6977; 95% CI = 1.4359-5.0685) and in female smokers at onset which almost half (47%) had rheumatoid nodules (p = 0.0014, OR = 2.9920; 95% CI = 1.5269-5.8631). The ratio of male smokers to the onset of disease with or without rheumatoid nodules is approximately 4: 1 (38%: 10%), which makes the relationship between smoking and the presence of rheumatoid nodules more evident to those who smoke at the onset of the disease (p = 0.0365, chi square = 4.3743).

We also met the same associations when we analyzed the subgroup represented by the Caucasian population. 46% of Caucasian smokers on the onset have or had rheumatoid nodules, and among non-smokers at onset, only 21% had this extraarticular manifestation (p = 0.0002, OR = 3.2105, 95% CI = 1.7264-5 , 9704).

Male smokers o the onset had anemia as much as non-smokers (47%) and men current smoker had anemia more frequenty than non-smokers (55% versus 42%), but not enough to achieve a statistically significant correlation between anemia and current smoker status in the men's subgroup (p = 0.5099).

Anemia is present in a large percentage amongst current smokers (54%) than non-smokers (35%), and ever smokers and smoker at onset are 50%, but statistically significant relationships between anemia and cumulation of tobacco can not be established.

Smoking and rheumatoid arthritis activity

Continuing the study, we looked at how smoking influences the disease activity expressed by DAS $_{28}$ composite index (Table XXIV).

Tabel XXIV. Degree of disease activity according to the status of smoker

DAS28ESR	ES	NS	SO	NSO	CS	CNS
Remission	11/85	25/164	6/65	30/184	2/32	34/217
LDA	20/76	20/169	16/55	24/190	3/31	37/214
MDA	39/57	72/117	31/40	80/134	17/17	94/157
HDA	26/70	72/117	18/53	80/134	12/22	86/165

The only statistically significant correlations were between low disease activity and ever smoker status (p = 0.0665, OR = 2.2237, 95% CI = 1.1307-4.373) and also smoker on the onset (p = 0.0195 , OR = 2.3030; 95% CI = 1.1435-4.6385).

We found the same relationships in the female gender subgroup, maintaining the proportions of women smoker on the onset and women ever smoker with low-activity rheumatoid arthritis and non-smoker women with the same type of disease (p = 0.0180, OR = 2.4490; 95%CI=1,1661-5,1433, respectively p=0,0103, OR=2,7469; 95%CI=1,2691-5,9458). Also smokers of Caucasians have low-activity disease (p = 0.0105, OR = 2.0593, 95% CI = 1.1120-3.8136).

Studying the activity of rheumatoid arthritis in heavy smokers at the onset, we noticed 51% with high activity compared to only 23% among non heavy smokers at onset (p = 0.0117, chi square = 6.3429). Thus, we can say that the big smokers have a more active disease.

We have further analyzed the inflammatory syndrome and the number of swollen joints, taking the values of the negative prognostic criteria of the Romanian Society of Rheumatology as reference values, considering them more suitable to follow the effects they have on the evolution of rheumatoid arthritis.

In ever smokers, the biological syndrome (ESR> 50 mm/h, CRP> 2.5 mg/dl) and SJC> 5 are present in double percent comparing with never smokers, values sufficient to achieve statistical correlation (p = 0.0063, OR = 2.2301, 95% CI = 1.2547 to 3.9637 for ESR, p = 0.0391, OR = 2 , 3676 ; 95% CI = 1.3051 - 4 , 2953 for PCR and p = 0.0014, OR = 2 , 5484; 95% CI = 1 , 4369 - 4 , 5197 for SJC) (Figure 40) .

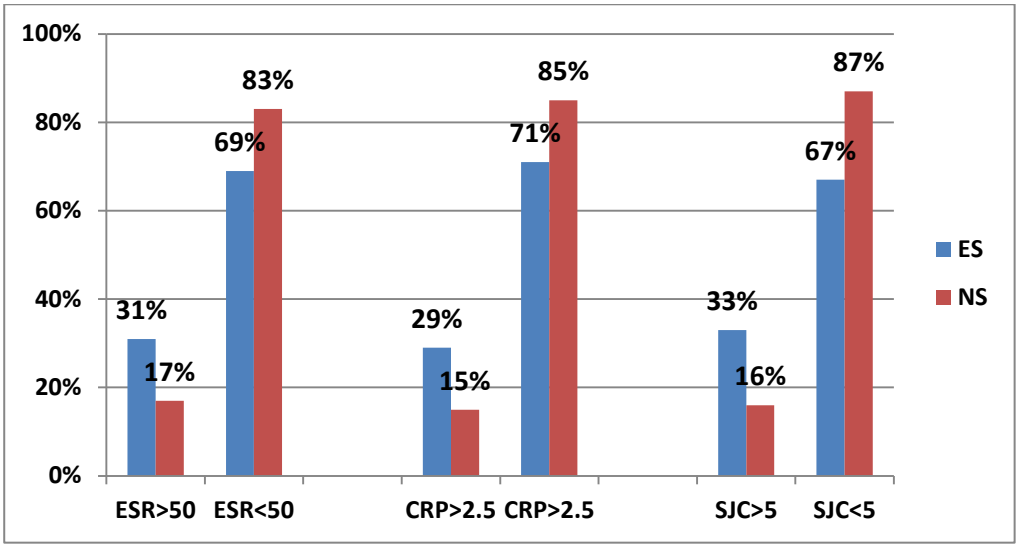

Figure 40. Correlations between disease activity indicators and smoking status in patients in the study group

Smokers on the onset have a higher percentage of CRP> 2.5mg/dl and SJC> 5 than non-smokers at the onset, high enough to create a statistically significant relationship between the two variables (p = 0.0391, OR = 1.9390 95% CI = 1.0337-3.6372 for PCR and p = 0.0392, OR = 1.8945, 95% CI = 1.0321-3.4776 for NAT). ESR> 50mm/h does not correlate with smoking on the onset (Figure 41).

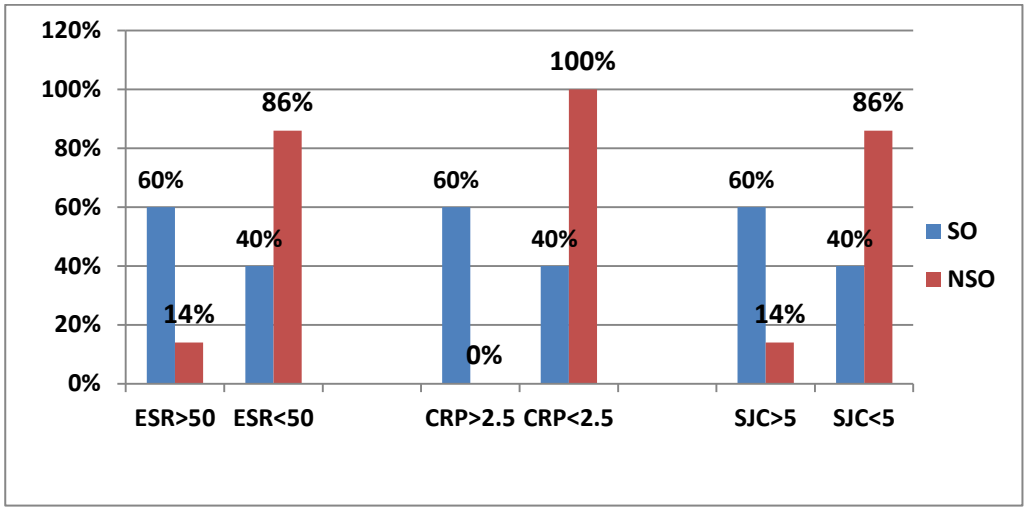

Figure 41. Correlations between indicators of disease activity and ever smoking status

Increased ESR values over 50mm/h occurred in 35% of smokers at onset (19% in non-smokers at onset), and 38% had over 5 swollen joints (20% non-smokers at onset). By performing the statistical analysis we obtained significant values in both situations (p = 0.0453, OR = 2.1927; 95% CI = 1.0167-4.7290 for ESR and p = 0.00184, OR = 2.4886; 95 % CI = 1.1663-5.3100 for SJC).

Caucasian race is associated with a greater number of swollen joints, with 37% of current smokers having more than 5 swollen joints at the time of examination (p = 0.0449, OR = 2.2906, 95% CI = 1,0190-5,1489).

We further investigated the effects of the amount of tobacco consumed on the biological syndrome of inflammation and joint inflammation. Heavy smokers do not associate a more intense biological inflammation syndrome, but the swollen joints are numerous. The association is statistically significant in the subgroups of the heavy ever smokers and heavy smokers at the onset (p = 0.0356 , chi-square = 4.4149 for the heavy ever smokers and p = 0.0143 , chi-square = 6,0009 for heavy smokers at the onset).

Smoking and treatment of rheumatoid arthritis

Regarding the treatment of patients in the our group, we tracked the number of synthetic disease modifying drugs (Methotrexate, Sulfasalazine, Hydroxychloroquine, Leflunomide) that they used throughout the course of the disease. As shown in Table XXV, there are no differences between smoker and non-smoking patients. pPercentages of patients who received 1, 2, 3 or 4 DMARDS among smokers and non-smokers are almost identical: 20% and 23% received a single DMARD, 17% and 19% received 2 DMARDs, 30% and 24% 3 DMARD, and 33% and 34% received 4 DMARDs.

Table XXV. The number of synthetic DMARDs depending on the status of ever smoker

		The number of synthetic DMARDs				Total
		1	2	3	4	
EVER SMOKER	1	19	16	29	32	96
	2	43	36	46	64	189
Total		62	52	75	96	285

Following the same on smoking and non- smokers at the onset, we found a statistically significant correlation between the consumption of 3 disease modifying drugs and the onset smoking status (Figure 42). 37% of the smokers on onset were needed 3 DMARDs, and among the non-smokers at the onset only 23% (p = 0.0242, OR = 1.9456, 95% CI 1.0908-3.4701).

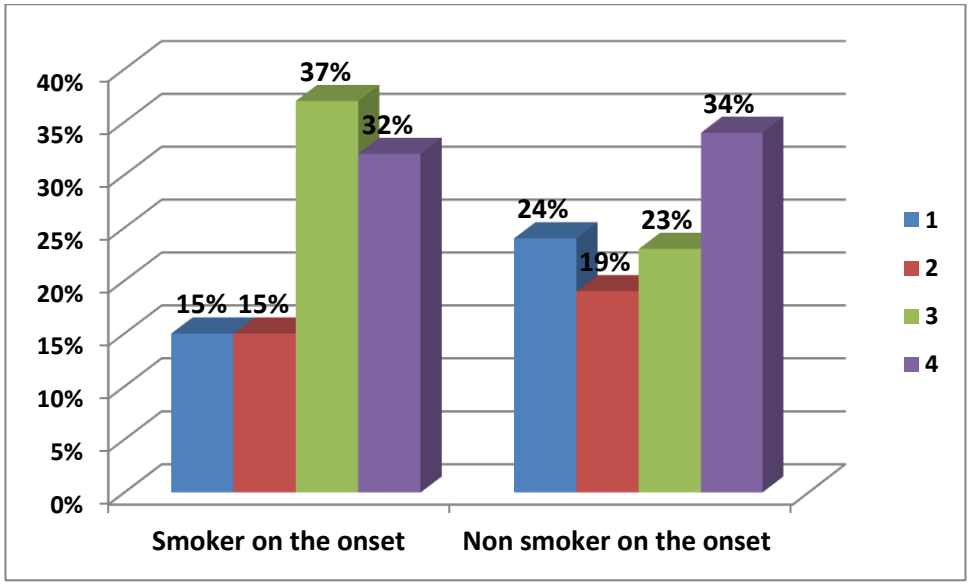

Figure 42. The number of synthetic DMARDs according to smoking status at onset

At time of emanimation, biological therapy is administered in 78 patients (27% of them). The share of biological therapy among smoker patients is higher than among non-smokers, however, the difference is not statistically significant (Figure 43).

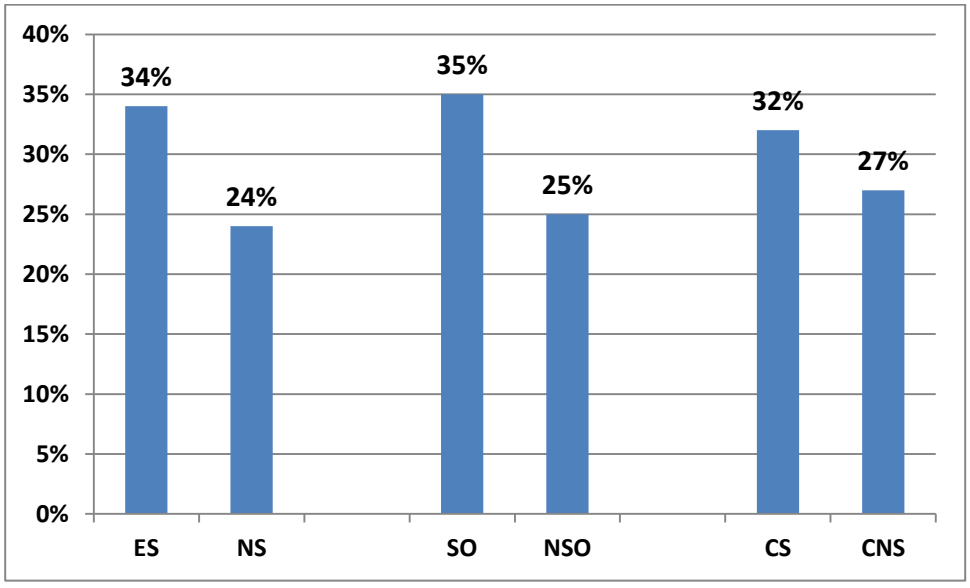

Figure 43. The share of biological therapy in patients in the batch depending on the status of the smoker

We have further analyzed how smoking habits modify the evolution of rheumatoid arthritis in patients undergoing biological therapy. We did not notice significant differences in seropositivity (rheumatoid factor, Ac antiCCP), clinical development (presence of ankyloses, erosions, extraarticular manifestations). The differences between smokers/non-smokers began to occur at the time when we analyzed the activity indicators of rheumatoid arthritis (biological inflammation syndrome, number of swollen joints). These indicators are used to monitor response to treatment. Their presence significantly higher percentage selected to smoking patients is an argument of it satisfactory response to biological therapy administered.

Of the acute phase reactants of inflammation, CRP has values above 2.5 mg/dl in 53% of smokers on onset who receive biological therapy and only 27% of non-smokers at the onset (p = 0.04938, chi-square = 3.8623).

ESR increase above 50mm/ h is more common in smokers on onset (53%) and current smokers (29%) than non-smokers on onset (26%) and current non-smokers (9%), with statistically significant correlations these variables (p = 0.0369, chi-square = 3.8623 for onset smoking and p = 0.04029, chi-square = 4.2057 for current smoking) (Figure 46).

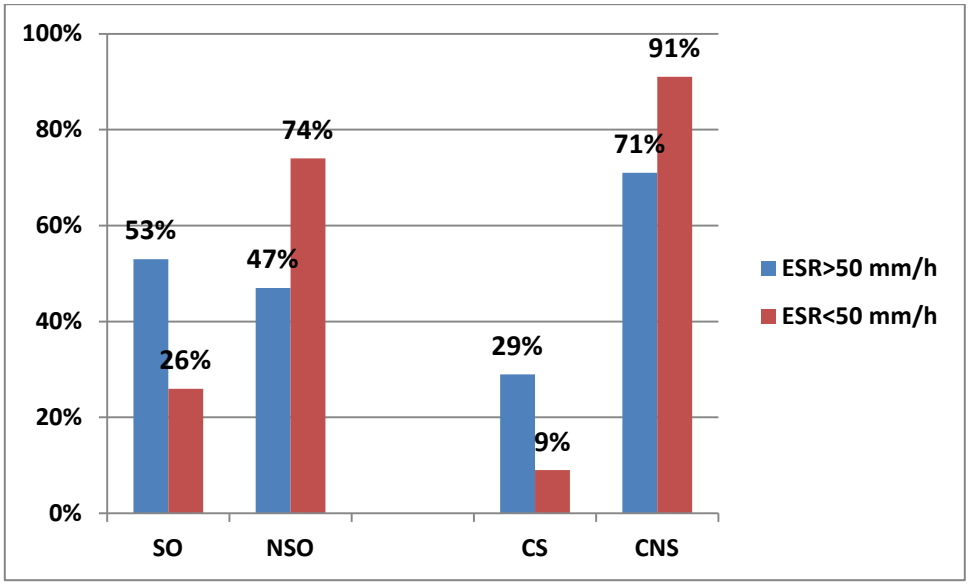

Figure 46. Correlations between smoker status and ESR increases in patients with RA treated with biological therapy

In the case of patients treated with biological therapy, swollen joints correlates with the status of the ever smoker, current smoker, smoker on the onset. The strongest correlation is smokers at onset, where

70% of the smokers on onset have more than 5 swollen joints compared to 25% of non-smokers at onset (p = 0.0016, chi-square = 9.9016) (Figure 47).

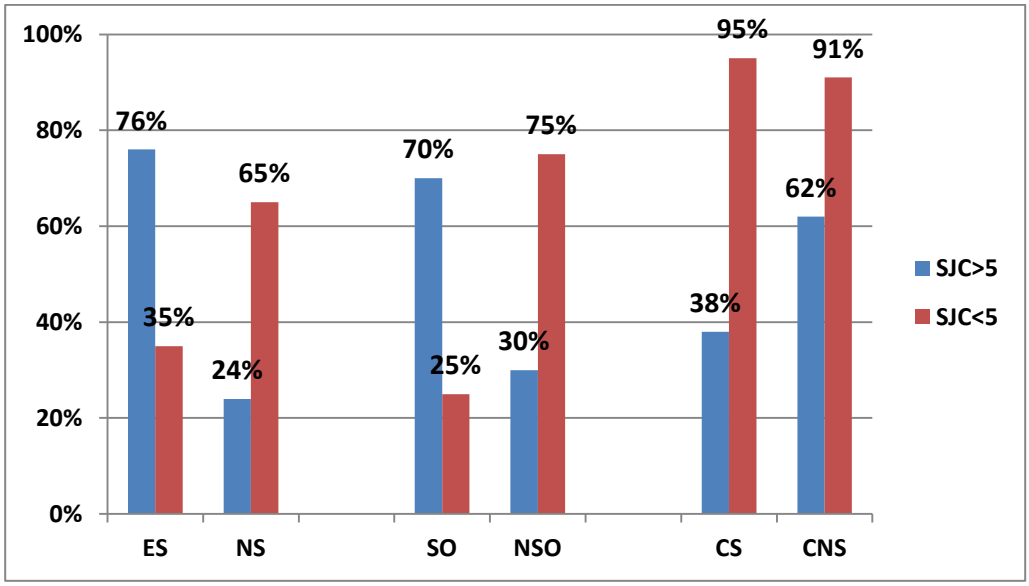

Figure 47. Correlations between smoker status and SJC in patients with RA treated with biological therapy

Conclusions

1. Patients in the study group are characterized by an average age of 62.02 years, 2/3 being in the 55-75 years age range, following the tendency of population aging , but also the tendency to increase the onset of rheumatoid arthritis. There are patients with old disease, established form, most seropositive, all having implications in the disease, patients with severely impaired functionality of joints, an impairment extra-articular more frequent and increased disease activity .
2. Patients are treated according to treatment guidelines, most of them (75%) receiving the first DMARD within the first 12 months of onset of symptoms, and the first choice was Methotrexate in 60% of patients.
3. Of the 285 patients in the study group , 33.7% have ever smoked, 24.9% have smoked at the onset of the disease, and currently smoke 11.9%. This prevalence is comparable to smoking prevalence among patients in the QUEST-RA multinational base, but much lower compared to other studies where the frequency of smokers exceeds 70 %.

4. The prevalence of smoking was higher among males (78%) than among women (26%). This difference is found in most populations studied, with the indication that it is not so great; on a national level, the difference between male and female smokers is 30%.
5. Smoker patients are significantly younger than non-smokers, a result different from the QUEST-RA multinational study, where smokers were ever older than non-smokers, but there was no difference in age distribution among male or female smokers, most being from the 55-65 age group. The fact that smokers are younger than non-smokers may be due to lower age at the onset of rheumatoid arthritis in our group, but also to the easier access to tabacco products in Romania than to other countries
6. The number of heavy smokers is higher among current smokers (41%), which is perhaps why they continue to smoke, with nicotine addiction being higher among them. Among the urban population at the onset of the disease, we encounter the most smoker smokers, the average PA being 26, with a range between 2 and 90 PA.
7. It was possible to establish a direct linear relationship of poor intensity between the age at the onset of rheumatoid arthritis and the number of packs/year smoked at that time, the amount of tobacco consumed prompting the onset of symptoms characteristic of rheumatoid arthritis.
8. Seropositivity (both for RF and antiCCP) correlated with smoking on the onset status, especially in Caucasian men. The presence of autoantibodies in the serum of the smokers on onset may be explained by the fact that they appear in the serum before the symptoms of rheumatoid arthritis occur, and there are studies that consider smoking to be the *trigger* for the production of these autoantibodies.
9. The role of *triggering* smoking in the beginning of autoimmune processes could explain that the age at onset of rheumatoid arthritis was about 5 years lower among smokers. In smokers, the onset was 8 years earlier than in non-smokers, possibly by reducing the protective role of smoking-related estrogens.
10. In subgroup analysis, a more severe articular affection was noted among male smokers, at the time of examination, they showed erosions and joint anchiloses with a significantly greater share. As we do not develop patients in evolution, we have not been able to determine the time of radiological changes. The protective role of smoking in the development of other inflammatory diseases explains why radiological changes have not been more severe in large smokers.

11. Current smokers have extraarticular manifestations with a significantly lower share compared to current non-smokers (59% versus 70%, p = 0.0471). These data are supported by numerous observational studies conducted in different populations and aimed at determining predictor factors for the appearance of extraarticular manifestations.
12. Following the occurrence of rheumatoid nodules we found correlations statistically significant between the status of current smokers and ever smoker (p = 0.004, p = 0, 0018), regardless of sex and selected especially Caucasians; this correlation is explained perhaps by the presence of rheumatoid factor and Anti-CCP is high in smokers at the onset of the disease .
13. The heavy smokers in the studied group had a high-disease disease, DAS_{28} in their case having significantly higher values than the rest of the patients. High disease activity among smokers is also supported by the presence of the biological inflammation syndrome and the number of swollen joints. The status of the smoker at the onset affects the negative prognostic factors (ESR ≥ 50mm / h, CRP ≥ 2.5mg / l, SJC ≥ 5), with statistically significant correlations between smoking and these factors. Our results differ from those from studies performed on patient cohorts in Europe due to the particularity of the studied group.
14. Smokers at the onset of rheumatoid arthritis needed several synthetic DMARDs along the course of the disease. The number of smokers on onset who were treated with biological DMARDs at the time of examination was higher than that of non-smoking patients, but not statistically significant.
15. Smoker patients responded less well to anti-TNF alpha therapy than non-smokers. This unsatisfactory response is expressed by higher values of disease activity indicators (SJC, ESR, CRP) in smokers who are receiving this disease-modifying therapy. In fact, as a result of numerous studies, smoking is considered a predictive factor of lack of response to anti-TNF alpha inhibitors.

Originality of the thesis

The topic of " Influence of smoking on the appearance and development of rheumatoid arthritis " is current and opportune especially due to the increase prevalence of rheumatoid arthritis, but also because of the impact this disease has on quality of life of patients who develop irreversible changes from the first years installing the condition.

Prevention of rheumatoid arthritis is a top priority, in 2016 EULAR launching the idea that there are some symptoms that it has formulated in the form of classification criteria that would raise the

suspicion of progressing to rheumatoid arthritis. Knowing the environmental factors that trigger autoantibodies and which hurry the first symptoms is very important for the primary prevention of rheumatoid arthritis.

The originality of the thesis consists in the fact that it realizes a premiere in the Romanian research for rheumatoid arthritis, namely the first study exclusively dedicated to the influence of smoking on the appearance and evolution of rheumatoid arthritis and which follows all aspects of the disease, from the appearance to the clinical, biological and radiological evolution, detailed smoking correlation, smoking habits of patients, the influence of smoking on the antibody titre characteristic for rheumatoid arthritis, the correlation of smoking with biological inflammation syndrome, the response to differentiated treatment on ethnicity and the amount of tobacco consumed in a comprehensive manner, extensively assessing the benefit of a complex, etiopathogenic and clinical approach.

Internationally, the study is valuable because it addresses this issue for a population with particular characteristics. This is also added to the fact that at the beginning of the research, the association between smoking and rheumatoid arthritis was a topic of topicality, and new studies are still being published, with new pertinent results.

The research presented in this thesis brings important data from the real life of patients with rheumatoid arthritis in Constanta County, and can open new perspectives on the diagnosis, monitoring and treatment of rheumatoid arthritis. At the same time, clear indication of the harmful role of smoking should cause rheumatologists, in collaboration with pneumologists, to create primary and secondary prevention programs that would help smokers with or without rheumatoid arthritis to give up much easier harmful habits.

SELECTIVE BIBLIOGRAPHY

1. Spector TD. Rheumatoid arthritis. Rheum Dis Clin North Am 1990; 16:513.

2.Gabriel S. Epidemiology of rheumatic diseases. In: Ruddy L, Harris E, Sledge C. (eds.) Kelley's Textbook of Rheumatology. 6 ed. Philadelphia, Pennsylvania: W. B. Saunders Company; 2001; 321-333.

3. Gerlag DM, Norris JM, Tak D. To prevention of autoantibody-positive RA: from lifestyle modification to preventive treatment. J Rheumatology. 2015; 55(4): 607-614.

4. Van Steenbergen WH, Aletaha D, Brouwer E, Beaart-Van De Voorde LJ, Codreanu C, et al. EULAR definition of suspicious arthralgia for progression to RA. Ann Rheum Dis. 2016; 0:1–6.

5. Liao KP, Karlson EW, Alfredsson L. Environmental influences on risk of RA. Curr Opin Rheumatol 2008; 21:279-283.