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THE BIOLOGICAL TREATMENT IN RHEUMATOID ARTHRITIS

Abstract

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Key words: rheumatoid arthritis, anti TNF-α agents, smoking, glucocorticoids, platelet indices

RHEUMATOID ARTHRITIS

1.1 ETIOLOGY

Rheumatoid arthritis (RA) is a multifactorial disease characterized by chronic destructive arthritis and multiple systemic manifestations, resulting from the interaction of several different factors which contribute to its occurrence and expression (1). The incidence of PR is about 95-150 new cases per 100,000 population per year and the prevalence rate is 1.7% for women and 0.7% for men (2)

The main risk factors for disease include genetic susceptibility, sex and age, environmental factors like smoking and infectious agents, hormonal status, socio-economic and ethnic factors.

In support of genetic factor is the fact of the disease concordance is grater in monozygot twins (14-24%) as compared to dizigotic twin (4%) (1). The stronger genetic association is to HLA class II histocopatibility antigens, the most common types associated with a high prevalence of disease is HLA-DR4 and HLA-DR1. The geneteic association is to those HLA-DR gene loci that code the “shared epitope” (SE)(1-4).

Other non-HLA genetic factors are: PTPN22, CTLA4, PADI4, IL10, TRAF1 /C5, STAT4 (3,5).

Sex : RA is a chronic inflammatory disease, with a higher occurrence rate in women, the female/male ratio being 2/1 to 4/1.

The most risk factor associated with RA is smoking (3,6). Numerous studies have shown that the duration and intensity of smoking is correlated with the risk of developing RA, and this risk remains even several years after smoking cessation (6). Smoking is a risk factor particularly in patients seropositive for RF (rheumatoid factor). Also increases the risk of developing ACCP (anti cyclic citrulline peptide antibodies) in patients with HLA-SE (3,6).

Infections have been studied in RA. Because the normal host molecule "looks like" a molecule on the offending organism that triggered the initial immune reaction- the phenomenon is called molecular mimicry. Some infectious organisms suspected of

triggering rheumatoid arthritis include viruses and bacteria (mycobacteria, streptococcus, Ebstein-Barr Virus, Parvovirus and rubella) (1-4).

1.2 PATHOGENESIS

The normal synovium outlines the inner cavity of the synovial joint. It consist of a linning layer (the region in direct contact with synovial fluid) and a sublinning layer. The linning layer is thickened to 2-3 layers. Synoviocytes are the predominant cell type in synovial membrane. The sublinnig layer include synoviocytes, adiposse tissue, blood vessels, nerves. Synovial cells are two types: A (synoviocytes like-macrofages-like) has a rich representation of organelles that are active, suggesting phagocytosis properties. Type B, known as the Fibroblast-like cells (FLC), has an abundant endoplasmic reticulum. It is involved in the synthesis of hyaluronate in synovial fluid (1-4,7).

Pathology in synovium rheumatoid represent a synergistic and complex interrelation between cells and their products. Resident cells, which represent "host tissue" are: synoviocytes A and B, endothelial cells and adipocytes. Cells that infiltrate synovial tissue are T cells, B cells, dendritic cells, macrophages, polymorphonuclear cells (PMN), natural killer cell, mast cells, thrombocytes, nurse-like cells, mesenchymal stem cells (3,8).

Cell communication occurs principally by cell to cell contact through cell surface molecule, or by soluble molecules called cytokines. Cytokies are small protein/glycoproteins 5-50 kDa with short half-life (minutes). They act as chemical messengers of the immune system allowing communication between innate and adaptive immunity during infection, inflammation, injury.

CD4+ T cells cytokines can be divided into Th1 and Th2 type cytokines, promoting cell mediated and humoral immunity respectively. There is a dysregulation in RA synovium, promoting Th1 cytokines with almost no Th2 cytokines being expressed, in turn inducing abundant proinflammatory cytokines by macrophages, fibroblast and endothelial cells (1-4).

The most relevant cytokines in RA are TNF, IL-1, IL-6.

TNF-α is one of the first cytokines secreted in the PR and it is considered to be the main trigger and enhancer of the inflammatory process.

TNF functions are pleiotropic and act in synergy with IL-1.

TNF actions are (9):

- increases expresion of adhesion molecule
- alters the normal procoagulant function of endothelium
- stimulates limphocytes, plays a role in the development of lymphoid tissue, induces maturation of dendritic cells and their migration to secondary limphoid tissue
- activates neutrophils and platelets
- induces fibroblast proliferation
- induces proinflammatory cytokines and matrixmetallproteinases

Previous research has highlighted the role of IL-1 in the pathogenesis of RA.

IL-1 family consists of three polypeptides similar in structure: IL-1α, IL-1β, which are agonists molecules with similar biological functions, and IL-1ra, an endogenous antagonist, which regulate, at least partially, the activity of IL-1α and IL-1β (10).

IL1 has the following roles (1-3):

- stimulates expression of adhesion molecules on endothelial cells and FLC
- stimulates angiogenic factors and neoangiogenesis
- stimulates the production of proteinases in chondrocytes
- activates osteoclast, promotes bone destruction
- stimulates the release of proinflammatory cytokines
- stimulates type B synoviocytes to proliferate

IL-6 is raised in serum and synovial fluid of RA patients. It has a role in activation, differentiation and proliferation of B lymphocytes, Ig synthesis, cytotoxic T lymphocyte differentiation and regulation of acute phase reactants in the liver (1-3).

1.3 CLINICAL MANIFESTATIONS

RA is a heterogeneous disease with no pathognomonic signs, symptoms or laboratory tests. The diagnosis of RA is facilitated by use of The American College of Rheumatology Criteria from 1987 – table 1(11). The sensitivity and specificity of the ACR 1987 criteria in early RA are low. During 2010 updated criteria were issued by the

ACR and the European League Against Rheumatism (EULAR) (12). The 2010 criteria are a score-based algorithm (table 2); an overall score of 6/10 is needed for classification of a patient as having RA.

Table 1 – The ACR Criteria for RA (1987)

i. Morning stiffness 1 hour

ii. Arthritis of 3 joints/joint groups

iii. Arthritis of the hand joints .

iv. Symmetry of arthritis

v. Rheumatoid nodules

vi. Rheumatoid factor (RF)

vii. Radiographic changes

* Criteria 1-4 should be present for at least six weeks. Patients fulfilling • 4 out of 7 criteria are classified as having rheumatoid arthritis.

Table 2 – the 2010 ACR/EULAR scoring criteria for Classification of RA

Group A – Joint involvement	Score
i. 1 large joint	0
ii. 2-10 large joints	1
iii. 1-3 small joints, with or without involvement of large joints	2
iv. 4-10 small joints (with or without involvement of large joints	3
v. >10 joints (at least one small joint)	5
Group B – Serology (at least 1 test result is needed for classification)	Score
i. Negative RF and negative ACPA	0
ii. Low-positive RF or low-positive ACPA	2
iii. High-positive RF or high-positive ACPA	
C – Acute-phase reactants	Score
i. Normal CRP (C-reactive protein) and normal ESR (erythrocyte sedimentation rate)	0
ii. Abnormal CRP or normal ESR	1
Group D – Duration of symptoms (self-reported)	Score
i. <6 weeks	0
ii. >6 weeks	1

Recurrent chronic inflammation leds to different degree of joint destruction and disability. Some patients have extraarticular disease that include rheumatoid nodules, vasculitis, Sjogren' syndrome, pulmonary manifestations, renal disease, bone and muscular manifestations (1-4,13,14). RA is associated with premature mortality, especially cardiovascular related.

Laboratory tests show inflammatory syndrome, haemathological and immunological changes (RF, ACCP). X-rays and other medical imaging techniques such as magnetic resonance imaging (MRI) and ultrasound are also used in rheumatoid arthritis (1-4,15,16).

1.4 TREATMENT

Pharmacological treatment of RA consists of symptomatic and disease modifying therapy.

- SMARDs (symptom modifyng antirheumatic drugs) include non steroidal anti-inflammatory drugs (NAISDs) and glucocorticosteroids (GCs)
- DMARDs (disease modifying antirheumatic drugs) include methotrexate sulfasalazine, leflunomide, gold compounds, penicillamine, antimalarials, cyclophosphamide, azathioprine, cyclosorine and biologics – anti TNF-α agents, rituximab, tocilizumab, abatacept, anakinra

NAISDs are the first drugs used in RA. They ameliorate the symptoms of the disease, without effects on the spontaneous course of the disease (17). They are administred as co-medication.

Glucocorticoids are widely used anti-inflammatory and immunosuppressive drugs for rheumatoid arthritis (RA) to control symptoms of pain and morning stiffness.In low doses, GCs has a proper role as a DMARDs (18). Glucocorticoids are highly effective for relieving symptoms in patients with active RA in doses of less than 10 mg/day. In severe RA, GCs are administrated in medium doses (20-30 mg prednisone per day or equivalent) or high doses. In RA, pulse therapy is applied to treat serious complications of the disease and to induce remission in active disease. Pulse therapy with schemes of 1000 mg of methylprednisolone intravenously for 3 days or 250 mg methylprednisolone for 5 days has been proven to be effective (19).

DMARD are chemically different compounds which influence the course of the disease. This group include drugs that rarely cause a real remission of the disease, they impove functional status and slow the destructive process in bone in cartilage (19).

DRUG	DOSAGE	SIDE EFFECTS
Methotrexate	7.5 to 25 mg / week,p.o, im, ,sc	gastrointestinal,hepatic,fibrosis,pulmonary, myelosuppression,alopecia,teratogenicity
Leflunomide	10-20 mg / day	digestive,liver, bone marrow toxicity
Sulfasalazine	2000-3000 mg / day	digestive,liver, bone marrow toxicity
Hydroxychloroquine	200-400 mg / day	Retinal toxicity, rash
Gold salts	50 mg / wk im	stomatitis,,myelosuppression
Azathioprine	50-150mg / day	myelosuppression,hepatotoxicity
Cyclophosphamide	1,5-2,5 mgkg day/ po 10-15 mg in 2-6 weeks kg/day	myelosuppression, hemorrhagic cystitis, infections,infertility,cancers
Ciclosporina A	2.5 to 5 mg kg / day	nephrotoxicity,hepatocytolisis

An association with DMARDs with different mode of action ideally results in additive or even synergistic effects, while potential adverse effects remain at the level associated with the dose of each component (19.22).

Biologics

In recent years, the discoveries in the domain of immunopathology combined with the progresses in biotechnology and molecular biology have enabled the introduction of biologic therapies possible (23).

The biologic therapies represent the usage of medicaments which have immune or genetic factors as a goal with the role of modulating the disease (23).

Biological agents include: anti TNF agents, monoclonal antibodies against CD20 specific B-cell antigen, and anti IL-6 blockers. Efficacy of biologics was shown in randomised clinical trials in patients with RA, both as monotherapy and in combination with DMARDs (24-32).

Anti TNF-α agents

Currently, there are three anti–TNF-α agents available for clinical use: infliximab, etanercept, adalimumab.

Anti TNF agents are indicated in patients with active disease (DAS28>5,1), in whom disease remains active despite the concurrent use of at least 2 conventional DMARDs for at least 12 weeks each, if one of them is MTX (excepting the cases in which MTX is not tolerated or contraindicated) (19).

Contraindications for TNF bloking agents are: severe, cronic infections, demyenlinating disease, heart failure, pancytopenia, malignicies, pregnancy and breastfeeding.

The adverse events associated with the use of TNF include infusion and injection reactions, infections, reactivations of latent tuberculosis, malignicies, drug-induced lupus, demyenlinating disease, worsening of preexisting heart failure (1-3,29).

Infliximab is a chimeric mouse-human monoclonal antibody composed of constant regions of human immunoglobulin (Ig) G1κ coupled to the variable regions of a high-affinity neutralizing murine anti–human TNF-α antibody. The resulting construct is approximately 70% human (1-3,23).

The typical initial dose of infliximab in RA is 3 mg/kg given as an intravenous infusion, followed by doses 2 and 6 weeks after the first infusion, then every 8 weeks thereafter. For patients who have an incomplete response, dosing may be increased up to 5 mg/kg, or the drug may be administered as often as every 4 or 6 weeks (19).

Etanercept is formed by the linkage of two soluble p75 TNF-R extracellular domains to the Fc portion of human IgG1. Etanercept binds both TNF-α and LT-ß with high affinity and specificity. Etanercept is administered by subcutaneous injection in doses of 25 mg twice weekly or 50 mg once weekly (1-3,23).

Adalimumab is a human anti-TNF IgG1 monoclonal antibody generated through repertoire cloning. Adalimumab neutralizes the biologic activity of TNF-α by binding with high affinity to the soluble and transmembrane forms of TNF-α and inhibiting the binding of TNF-α with its receptors. Adalimumab is administrated as 40 mg subcutaneously every other week (19).

Tocilizumab

Tocilizumab is a humanized IgGl mAb that binds with high affinity to IL-6R. The recommended dose of tocilizumab is 8 mg/kg administered as a single 60 minute intravenous infusion every 4 weeks (19). The adverse effects include infection, transient increases in transaminases and cholesterol and neutropenia (3).

Rituximab

Rituximab is a chimeric mouse-human monoclonal antibody directed against the extracellular domain of the CD20 B-cell antigen. It consists of an Ig G1 Fc constant region and variable light and heavy small regions of the murine antibody fragments, which are reactive with human CD 20 (1-3). Rituximab is administrated at a dose of 1000 mg repeated at 2 weeks. Repeated doses are indicated at 6-9 months (19). Most RA occur during infusion - fever, headache, myalgia (30.31). Administration of corticosteroids decreases the incidence and severity of infusion reactions (19). Other side effects - increased risk of opportunistic infections, worsening of HBV infection.

SPECIAL PART

Introduction

Two concepts have recently emerged in the treatment of rheumatoid arthritis (RA) - "window of opportunity" and "tight control of disease". They refer to a period in which the response to the therapy is effective, translating into disease remission or major benefits. The introduction of biological agents in the therapeutic arsenal of RA, resulted in fundamental changes for the better, in terms of quality of life in patients with RA. However, there is a significant percentage of patients unresponsive to treatment, with poor response, or with a good response initialy but with lost of effectiveness over time, setting up drug resistance. In recent years, new biological agents with other mechanisms of action are available to treat patients with RA, increasing the number of therapeutic options in patients who responded inadequately to anti-TNF-α therapy. Rituximab is a monoclonal antibody against CD20 B lymphocyte.

Rituximab which is used in patients with severe disease has led to significant benefits, supporting its use in combination with DMARDs in patients unresponsive to anti-TNF-α medication. In this study I evaluated the biological treatment duration and response to treatment, in patients who have changed biological therapy.

The introduction of biological agents has led to the reevaluation of existing therapies in patients with RA. Although new antirheumatic drugs have a well established effect on synovitis and radiological progression, the association of corticosteroids provides clinical benefits and delays radiological progression.

It is known that glucocorticoids (GC) are used as soon as possible as bridging therapy to control the disease. The use of GC can leed to well known adverse events such as osteoporosis, hypertension, diabetes, infections, gastrointestinal disorders, eye disorders (cataracts, glaucoma), skin changes etc. The current view on these drugs is that they are indispensable. They should be administered as much as necessary but as little as possible. Therefore, I tried to determine the impact of TNF-α blockers on the use of GC, in order to decrease or discontinue the corticosteroid therapy.

It is known that smoking is a major risk factor for RA. Smoking is associated with production of RF (Rheumatoid Factor) and ACCP (Anti-Cyclic Citrullinated Peptide Antibodies) and extraarticular manifestations such as rheumatoid nodules and vasculitis. Recent studies have shown that smoking influences the response to treatment. In this paper I try to evaluate treatment response with TNF-α blockers in two groups of patients with RA, smokers and nonsmokers respectively.

Platelets role in the pathogenesis of RA is well known. Recently, an increasing interest is given to platelet indices: MPV (mean platelet volume) and PDW (platelet distribution width). Clinical studies showed that platelet indices are related to cardiovascular and metabolic diseases. These relationship was studied in acute coronary syndromes, myocardial infarction, peripheral ischemia syndrome of the lower limbs, stroke, hypertension, atrial fibrillation, dyslipidemia, diabetes and metabolic syndrome. There is conflicting evidence regarding these data in patients with RA. In this study, I tried to evaluate the influence of anti TNF-α agents on platelet indices and the relationship between these indices and disease parameters.

Objectives

We conducted four retrospective studies in patients diagnosed with RA according to the ACR (American College of Rheumatology) criteria, admitted between January 2009 - June 2011 in Cantacuzino Hospital, in Bucharest. The data were collected starting from the initiation of the first biological agent.

We studied the following:

-the influence of smoking on response to treatment with TNF-α blockers

- biological treatment duration and treatment response in patients who switched biological treatment

-the impact of anti TNF-α agents on the use of glucocorticoids

- the influence of TNF-α blockers on platelet indices

2.2 INFLUENCE OF SMOKING ON RESPONSE TO TREATMENT WITH ANTI TNF-α AGENTS IN PATIENTS WITH RHEUMATOID ARTHRITIS

Smoking is an important environmental factor in the development of RA. Recent studies have shown that smoking influences the response to treatment (33-36).

The **objective** of this study was to evaluate the influence of smoking on response to treatment with anti TNF-α in a group of patients with RA.

Method

We included 78 patiens fulfilling 1987 ACR criteria. Patients were enrolled into two groups: group 1 - non-smokers and group 2 - smokers or former smokers. For each patient demographic, clinical, laboratory data as well as data regarding therapy were completed. We also noted the presence of diabetes mellitus (DM), hypertension (HTA), ischemic coronary heart disease (IHD), dyslipidemia, lung disease (chronic bronchitis, pulmonary fibrosis), rheumatoid vasculitis, peripheral arterial disease and osteoporosis. Data were recorded before the initiation of biological therapy, at 3 months, 6 months and one year after treatment initiation. Disease activity score (DAS28) was noted initially and after one year of treatment.

Measurements are presented as mean values ± standard deviation. Variables were compared using Student tests, ANOVA, Chi.test. All statistical analyzes were performed using SPSS 13.0 for Windows (SPSS, Inc.., Chicago, Illinois). Values of p less than 0.05 were considered statistically significant.

Results

Seventy-eight patients were enrolled, including 40 non-smokers and 38 with a history of smoking. Of the latter, 34 are smokers and 4 are former smokers.

A group of 51 patients were treated with infliximab (22 smokers and 29 non-smokers), 18 with etanercept (10 smokers and 8 non-smokers) and 9 with adalimumab (6 smokers and 3 non-smokers).

Smoking patients had more frequently a high educational level, were more frequently men - 55% vs. 15% non-smoking ($p = 0.001$). They were more often RF seropositive (89.4% vs. 68% ($p = 0.05$) and more frequently had rheumatoid nodules

(RN) (29% vs 10% (p = 0.04)). Mean age and mean disease duration were similar in both groups (p = ns). Two smoker patients had rheumatoid vasulitis.

ESR (erythrocyte sedimentation rate), CRP (C-reactive protein) and DAS28 (disease activity score) were similar in smokers and non-smokers patients initially. Inflammatory markers decreased significantly in both groups after 3 month and 6 month of treatment, but there were not statistically significant differences between smokers and nonsmokers (p =ns).

ESR and CRP were significantly higher in smokers than in non-smokers after one year of treatment (ESR=37.3±27mm/h vs de 24.7±19mm/h, 18.9±18mg/l vs 9.2±9mg/l, respectively, p=0,03). Smokers had a higher number of swollen and tender joints than nonsmokers at one year, but these difference were not significant ((4.5±6,6 vs 3.6±4 and 3.1±3,5 vs 2.5±3.8 respectively; p>0.05). DAS28 was 4±1.3 in smokers versus 3.4±1 in non-smoker patients (p=0.03). DAS28 decrease was significantly higher in nonsmokers (p = 0.02).

Eight smokers (21%) were with active disease (DAS>5,1) at one year versus 4 (10%) non-smoker patients and 5 (13%) smokers were in remission (DAS<2,6) at one year.versus 12 (30%) non-smoker patients.

Smokers used more frequently a combination of DMARDs (15 versus 11), changed the associated medication more frequently (9 vs. 5) and used more frequently antiinflammatory drugs / analgesics daily (6 versus 2) and glucocorticoids (GC) (24 versus 17) than non-smokers, although the differences were not statistically significant (p = ns).

Regarding side effects, three non-smokers patients and one smoker patient had pulmonary tuberculosis after treatment with infliximab, and two non-smoker patients had an allergic reaction to infliximab.

Discussion

In our study, smokers and nonsmokers had similar values of ESR, CRP, DAS28 initially. As other authors have shown in previous studies, smokers were more often RF seropositive and had more frequently rheumatoid nodules suggesting persistent immune activation in smokers (37,38).

The literature have shown that smokers have a poor response to TNF-α-blocking therapy in comparison with non smokers (33-36).

In our study, although smokers had slightly elevated ESR and CRP than nonsmokers after 3 and 6 months of treatment, differences were not statistically significant (p = ns). In our study, smokers had higher NAD and NAT (p = ns) and also higher inflammatory markers ($p<0.05$) than nonsmokers which may sugest a possible increase in inflammation level in smokers.

The literature have shown that smokers have a higher need for biological agents and higher doses of DMARDs (33.34). The authors suggest that smoking decreases the potency of these drugs and smokers need higher doses to control the disease (33.34). In our study, smokers have poorly responded to biologial treatment than non smokers and they installed resistance to treatment faster than nonsmokers, which led to medication switch. Smoking influenced the response to treatment with conventional DMARDs (smokers have changed more frequently associated remitting medication and used more frequently a combination of DMARDs).

A number of possible mechanisms may be considered to explain our findings (33). One possibility is that the association with poor response in smokers is due to an increased frequency of RF and anti-cyclic citrullinated peptide (CCP) autoantibodies in smoker patients (33). Other possibilities include an alteration in the pharmacokinetics of TNF antagonists in smokers, for example by interference with absorption, or more rapid clearance of drug .

In our study, smoker patients installed quickly resistance to treatment with TNF-α blockers than nonsmoker patients - treatment duration was less in smokers than non-smokers (32 ± 21 months vs 42 ± 22 months, p = 0.05) .

Regarding side effects, in our study, although we found a higher rate of pulmonary TB and allergic reactions to infliximab in smokers, other authors have described a similar rate of adverse events in smokers and non-smokers (36).

Conclusion

Smokers have responded poorly to treatment with anti TNF-α biological therapy when compared to non-smokers. They have installed more quickly treatment resistance, which had led to change medication.

Smoking influenced the response to treatment with conventional DMARDs (smokers have changed more frequently the associated remitting medication and have used more frequently combinations of DMARDs).

Smokers had a tendecy to use higher doses of GC than nonsmokers after a year of treatment with anti TNF agents.

It is essential to inform patients about the role of smoking in the development of RA and treatment evolution.

Smoking cessation should be recommended for all smokers with RA.

2.3 EVALUATION OF BIOLOGICAL TREATMENT DURATION IN A GROUP OF PATIENTS WITH RHEUMATOID ARTHRITIS

Treatment with anti TNF-α biological agents has substantially improved the therapeutic expectations in RA patients. Despite their effectiveness, there is a significant proportion of patients who discontinued treatment due to ineffectiveness, side effects or loss of effectiveness over time.

The **objective** of this study is to evaluate the biological treatment duration and response to treatment in patients who switched to biological treatment.

Methods

The retrospective study included 90 patients fulfilling 1987 ACR criteria. Demographic data were recorded, biological therapy duration (from the beginning of biological treatment to time of inclusion in the study), drug persistence (at time of initiation of treatment until biological change) disease activity before the initiation of the first, the second and third biological agent and 6 months after treatment, evaluated by DAS28 score.

Measurements are presented as mean values ± standard deviation. Variables were compared using Student tests, ANOVA, Chi.test. All statistical analyzes were performed using SPSS 13.0 for Windows (SPSS, Inc.., Chicago, Illinois). Values of p less than 0.05 were considered statistically significant.

Results

Seventy percent of patients were women, mean age was 55.9 ± 11 years, mean disease duration 12.7 ± 6.6 years. Sixty six patients (73.3%) followed treatment with infliximab, 15 patients (16.7%) with etanercept and 9 patients (10%) with adalimumab. DMARDs were associated to biological therapy in 85 patients (94%).

Treatment duration was 36.9±23 month for infliximab, 28.5±19 month for etanercept and 27.4 ± 18 for adalimumab.

Treatment duration was higher in non-smokers than in smokers (42 ± 22 vs. 31 ± 18, p = 0.05). We excluded patients that have changed treatment because of side effects.

Drug survival was 30,6±21 month.

Drug survival was 35±21 month in patiens who have changed because of ineffectiveness and 15±12 month in patients who have changed because of adverse events (p <0.001).

Continuation rate for the first biological agent was 82%, 66% and 41% at 1 year, 2 years or 3 years respectively.

Treatment duration with the first biolgical agent was significantly higher in RF seronegative patients than in seropositive patients (p <0.001).

A number of 29 patients (32%) experienced adverse events, 23 to infliximab, 3 to adalimumab and 3 to etanercept. The most common adverse events were allergic reactions (4), skin reactions (4), infections (8) and pulmonary TB (6). Other side effects were the presence of 2 cases of antinuclear antibodies (ANA), 1 case of anti dsDNA, 1 case of pleural effusion, 2 cases of severe hypotension, and 1 case of headache, dizziness.

Patients who have associated GC were older (57.8 ± 11.6 years versus 52.5 ± 11.4 years, p = 0.05).

Fifty patients (55%) changed biological treatment. Thirty (60%) patients changed with rituximab and 20 (40%) patients changed with another anti TNF agent.

Treatment duration of the second biological agent was 20.7 ± 13 months. Ten patients have changed the second biological treatment: 7 patients have changed with rituximab: 3 etanercept,/rituximab, 4 adalimumab,/rituximab and 3 with another TNF blocker.

For people who have changed the second biological treatment, treatment duration was 16 ± 12 months (15 ± 8 months for rituximab and 20 ± 19 months for anti-TNF, p = ns), lower than the first and second biological agent.

DAS28 decrease was greater in those who have changed biological treatment with rituximab than those who have changed to another anti-TNF ($p < 0.05$).

Discussion

In our study, more patients received treatment with infliximab than etanercept and adalimumab. These differences are due to the fact that infliximab was the first anti-TNF in use. Treatment duration was similar for the three anti TNF agents used. Previous studies reported different results regarding persistence to treatment. Some authors have reported a greater remanence to treatment to infliximab, others to etanercept or adalimumab and other authors reported a similar persistence to treatment for the three TNF blockers (39-43). These differences could be due to different characteristics of the study groups, differences in prescription criteria at inclusion, different costs, differences in the criteria for limitation of treatment or medication related.

In our study, the first biologic treatment duration was lower in the FR positive ($p < 0.001$), which is probably due to a more aggressive disease in these patients.

In our study, the first biologic treatment duration was lower in smokers than in non-smokers ($p = 0.05$).

As other authors have shown in previous studies (44), mean age was higher in patients following treatment with AIS ($p = 0.05$).

The prescription of GCs was more frequent in older patients (possibly related to the tendency to be less aggressive with DMARDs because of their higher comorbidity).

Drug persistence of the first biological agent was lower in those who have changed the treatment due to adverse event than in those who have changed because of ineffectiveness ($p < 0.001$). The most common adverse events were allergic reactions to infliximab, infection and pulmonary TB (1-3.45). In our study, patients who experienced side effects were more frequently older women (probably associated with an increased number of comorbidities) and FR seropositive ($p < 0.05$).

Conclusions

Duration of therapy (from the initiation of biological treatment to inclusion in the present study) for the first anti TNF-α was similar between the three agents.

Duration of treatment for the first biological used was higher in patients with rheumatoid factor negative and non-smokers.

Persistence to treatment (at time of initiation of treatment until biological change) was similar in the three anti-TNF-α agents.

Side effects and inefficiency were the most important reasons to change the biological treatment. The most common side effects are allergic reactions, injection site skin reactions, infections and pulmonary tuberculosis. Of biological therapeutic agents, infliximab was frequently associated with adverse reactions to perfusion and tuberculosis.

Decrease of the score DAS28 was greater in those who switched to rituximab than those who switched to another anti-TNF-α.

Duration of the second and third biological agent was lower compared to the first anti-TNF-α.

2.4 THE EFFECT OF TNF-α BLOCKERS ON GLUCOCORTICOIDS USE IN PATIENTS WITH RHEUMATOID ARTHRITIS

Recent studies have shown that treatment with TNF blockers allow decrease the corticosteroids (GC) doses in most patients (46-48).

Our **objective** was to determine the impact of anti-TNF-α agents on GC use in RA patients.

Method

The retospective study included 64 patiens treated with biological therapy (anti TNF) and oral GC. Oral GC use were recorded at six-month and 1 year intervals and converted into prednisone equivalents. Usually, treatment discontinuation with AIS (antiiflamator steroids) was considered dose 0.

Measurements are presented as mean values ± standard deviation. Variables were compared using Student tests, ANOVA, Chi.test. All statistical analyzes were performed

using SPSS 13.0 for Windows (SPSS, Inc.., Chicago, Illinois). Values of p less than 0.05 were considered statistically significant.

Results

Forty-nine (76.5%) patients were women, 52 (81%) were RF seropositive, mean age was 57,34 ± 10,58 years, mean disease duration was 12 ± 6 years. Twenty-two (34%) patients were smokers.

Decrease of prednisone doses after 6 months and 1 year after treatment with anti-TNF biologic was statistically significant ($p < 0.05$). Initial prednisone dose was 9.6 ± 4 mg, after 6 months 7.4 ± 3.6 mg and after a year 6.4 ± 4.5 mg.

Prednisone dose was significantly higher in smokers than in non-smokers after 1 year of biological treatment (8.1 ± 4 mg versus 5.8 ± 4 mg, $p < 0.05$). Men used higher doses of GC than women after 6 months of treatment (9.7 ± 3 mg versus 6.4 ± 3 mg, $p < 0.05$) and after a year of treatment (9.3 ± 3 mg from 5.9 ± 4 mg, $p < 0.05$).

Thirty-one patients (48%) decreased prednison intake and 5 (7%) discontinued the tratment with GC; 8 (12%) increased prednison intake; the remaining patients were on constant dosage after six months of biological tratment. Thirty-seven patients (57,8%) decrease prednison intake and 7 (10,9%) discontinued the treatment with GC; 10 (15%) increased prednison intake; the remaining patients were on constant dosage after 1 year with biological tratment.

Discussion

Lower dosages of GC are given in combination with DMARDs and other drugs such as analgesics and NSAIDs in order to control disease activity and symptoms. This therapeutic approach has the rationale that a combination of drugs with different mechanisms of action results in additive or synergistic effects, while potential adverse effect remain at a level associated with the dose of each component (49). For GC treatment, this means that the more effective the treatment is with DMARDs, the lower the GC dosages.

Previous studies have shown that treatment with TNF-α blockers can reduce GC dosage in RA patients (46-48). Our study also showed a significant decrease of prednisone dosage after 6 months and 1 year of treatment with TNF-α blocker. The literature have shown that smokers use more frequently GC than non smokers (34). In our

study, smokers have used higher doses of corticosteroids compared with nonsmokers ($p < 0.05$) after 1 year of biological treatment.

Conclusion

Prednisone doses decreased after 1 year of treatment with anti TNF agents (9,6 initially to 6,4 mg after 1 year).

In our study, after 1 year of treatment with anti-TNF-α, about 10% of patients discontinued treatment with GC and about in half of pationts dose reduction was possible, that can reduce long-term risk related to comorbidity in patients with RA.

Men have used higher doses of GC than women after 6 months and 1 year of treatment with anti TNF-α agents.

In our study, smokers have used higher doses of GC after 1 year of treatment with TNF-α blocker compared with nonsmokers (8.1 ± 4 mg versus 5.8 ± 4 mg, $p < 0.05$).

Biological treatment with anti TNF-α agents allow dosage reduction or discontinuation of GC treatment.

2.5 THE EFFECT OF ANTI TNF-α AGENTS ON PLATELET PARAMETERS IN PATIENTS WITH RHEUMATOID ARTHRITIS

Platelets role in the pathogenesis of RA was already demonstrated. Today a new interest is given to platelets indices MPV (mean platelet volume) and PDW (platelet distribuition width). It was shown that MPV is inversely related to rheumatoid inflammation, although data are controversial (50-55).

The Objective of this study is to evaluate the effect of TNF-α blockers treatment on platelet indices (MPV and PDW) in RA patients naïve to anti-TNF.

Method

We performed a retrospective study on 51 consecutive patients fulfilling 1987 ACR criteria for RA. The demographic, clinical, laboratory, and medication were noted. We also noted the presence of ischemic coronary artery disease (CAD), hypertension, dyslipidemia and diabetes mellitus (DM). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were measured as markers of inflammation also disease activity

score, DAS28 (Disease Activity Score) 28. We noted the platelet indices MPV and PDW. Data were analyzed before the initiation of the biological treatment and after 6 months follow up.

Measurements are presented as mean values ± standard deviation. Variables were compared using Student tests, ANOVA, Chi.test. Pearson's correlation analysis was used to explore the relationship between parameters. All statistical analyzes were performed using SPSS 13.0 for Windows (SPSS, Inc.., Chicago, Illinois). Values of p less than 0.05 were considered statistically significant.

Results

Thirty seven patients (72.5%) were women, mean age was 53.39 ± 12 years, mean duration of disease was 11.4 ± 6.6 years. Thirty-nine patients (76.4%) were rheumatoid factor (RF) seropositive and 12 (23.5%) RF seronegative.

Thirty-five patients (68.6%) were treated with infliximab, 14 patients (27.5%) with etanercept and 2 patients (3.9%) with adalimumab. Combined therapy with DMARDs (alone or in combination) remained stable during the study - methotrexate, leflunomide, sulfasalazine, imuran, hydroxychloroquine.

Nineteen patients (37.2%) had hypertension, 11 (21.5%) had CAD, 20 (39.2%), dyslipidemia and 4 (7.8%) type II diabetes mellitus.

Before the initiation of anti TNF-α treatment, patients had elevated DAS28, ESR and CRP (6.05 ± 1.1, 51±22mm / h and 45 ± 22 ± 44 mg / l respectively). The average number of platelets was 364.56 ± 120.4 x 103 / ml, MPV value 8 ± 1.2 fl (normal values 0.4 to 10, 4 fl) and PDW value 16.49 ± 1% (normal values 12 to 16.5%).

In all patients, DAS28 correlated with ESR ($r = 0.487$, $p < 0.001$) and CRP ($r = 0.540$, $p = 0.001$), ESR was correlated with CRP ($r = 0.830$, $p < 0.001$). Platelet counts correlated with inflammation intensity reflected by ESR ($r = 0.289$, $p = 0.04$). Platelet counts correlated negatively with PDW ($r = -0.456$, $P = 0.001$). MPV and PDW were not correlated with inflammatory markers or disease activity in the total group of patients. Fourteen patients (27.4%) had reactive thrombocytosis. ESR was significantly higher than in patients without thrombocytosis ($p = 0.01$) and PDW was lower ($p = 0.002$). In the subgoup of patients with reactive thrombocytosis, MPV correlated with CRP marginally significant ($r = 0.99$, $p = 0.051$).

Platelet histogram showed elevated values of PDW (PDW average = 16.49 ± 1%). Twenty patients (39.2%) had PDW values over 16.5% - reflecting increased platelet activation in these setting. Among patients with elevated PDW, 2 patients were diabetic, 10 patients with hypertension, 3 patients with CAD and 5 patients with dyslipidemia.

Inflammatory markers decreased after 6 months of treatment with anti TNF agents (ESR initially 51 ± 22mm / h; ESR after 6 months (ESR6l) 26 ± 19 mm / h, p <0.001, CRP initially 45 ± 44 mg / l; CRP after 6 months (CRP6l) 8.7 ± 14 mg / l, p <0.001). VSH6l and CRP6l has correlated (r = 0.832, p <0.001). Disease activity decreased to 6 ± 1 to 3.8 ± 1.1 (p = 0.001) after treatment.

The platelets count decreased significantly from 364.56 ± 120 x103 / ml to 304.9 ± 71 x103 / ml (p <0.001) after treatment. MPV increased significantly (MPV initialy 8 ± 1.2 fl, MPV after 6 months (MPV6l) 8.8 ± 1.6 fl, p <0.001). MPV after 6 months (MPV6l) was negatively correlated with platelet count after 6 months (r = -0.388, p = 0.005), reflecting the inverse relationship between platelet count and mean platelet volume previously observed by other authors. MPV did not correlate with VSH6l, CRP6l or DAS28 after 6 months (DAS28 6l) in the total group of patients.

No statistically significant differences were noted after treatment regarding PDW in the entire group of patients (initial PDW = 16.4 ± 1%, PDW after 6 months (PDW6l) - 16.44 ± 1.2%, p = ns).

After 6 months of treatment, PDW6l was positively correlated with MPV (r = 0.619, p <0.001) and negatively correlated with platelet count after 6 months (r = -0.304, P = 0.03).

Six patients had elevated MPV after treatment (one patient with BCI, three with dyslipidemia and were two patients with hypertension).

In hypertensive patients PDW decreased (16.7 ± 1.3% initially to 16.1 ± 1.2% (p = 0.055) after 6 months of treatment. Initially, 10 hypertensive patients (52%) had PDW at the upper limit of normal values, their number decreased after treatment - 6 patients (31%). Among patients with dyslipidemia, 3 had elevated MPV and 6 patients had PDW elevated after biological treatment.

Discussion

In our study, an increase of MPV after 6 months of treatment with TNF-α blockers was observed. It is known that larger platelets are more active, releasing a wider variety of proinflammatory and thrombotic agents. There are contoversial data regarding MPV in pacients with RA. Milavanovic et al. reported lower MPV, the CRP, IL6 and platelets at 2 years after treatment (53), questioning the inverse relationship between platelet count and MPV observed by other authors in PR (56) and IBD (57). Kisacic et al. reported low levels of MPV in patients with RA compared with osteoarthritis patients. These values were significantly higher after treatment, but remained lower than the control group (52).Yazici et al. reported higher MPV values in patients with RA. These values correlated with the DAS28 and decreased after treatment with conventional DMARDs and TNF-α blockers (55). On the other hand, Gasparyan et al. have shown that smoking and intensity of inflammation (ESR) were independently associated with low MPV, while hypertension was associated with increased MPV (58). Recently, Jurcut et al. reported low MPV and elevated PDW in RA patients. PDW correlated with fibrinogen, but not with ESR or CRP (51).

The observed discrepancies, most probably reflect time-dependent changes of MPV, and its dual pathophysiological role in RA (i.e., involvement in inflammation and thrombogenesis) (50). Overproduction of pro-inflammatory cytokines and acute-phase reactants can suppress size of platelets by interfering with megakaryopoiesis with subsequent release of small size platelets from the bone marrow (50). A smaller MPV could reflect an accelerated maturation and a shorter life in active RA (54.59.60). This has not been demonstrated in other chronic inflammatory diseases as IBD (57.60). We can say tat that short life of platelets is a feature of PR (60).

Another possible explanation of the decreased volume of circulating platelets in active RA relates to the intensive consumption of large platelets at sites of inflammation (vascular wall and synovial membranes)(50,60). There is another less probable explanation regarding increased platelets / MPV reduction (61) and disease activity in RA - thrombocytosis is a feature of chronic bleeding and in RA could be secondary to occult gastrointestinal bleeding caused by NSAIDs, especially in patients with active disease (60).

In our study MPV increased after 6 months of treatment. Platelets, disease activity and acute phase reactants decreased after treatment. MPV increased after 6 months and negatively correlated with platelet count ($p = 0.005$) which corresponds to literature data (50).

Platelet counts correlated negatively with the PDW initially ($r = -0.456$, $P < 0.001$) and after treatment ($r = -0.304$, $P = 0.03$). MPV positively correlated with PDW ($p < 0.001$) after treatment. In our study MPV did not correlate with disease activity or inflammatory markers. In patients with reactive thrombocytosis, MPV was correlated with CRP – marginally significant ($p = 0.051$).

Our results complete the literature data regarding MPV in patients with rheumatoid arthritis and the effect of rheumatic treatment on MPV in these patients (50.52). In our study, the conditions associated with an increased risk of thrombotic events (diabetes, dyslipidemia, hypertension) influenced platelet indices, which is in line with available data (62-64).

Conclusion

In our study, after treatment, platelet count decreased and MPV increased and was negatively correlated with platelet count.

MPV did not correlate with inflammatory markers or disease activity

Our results complete the literature data regarding changes of MPV in conditions associated with a high degree of inflammation.

In our study, the conditions associated with an increased risk of thrombotic events (diabetes, dyslipidemia, hypertension) influenced platelet indices.

Usefulness of platelet indices, which are today analyzed by routine automated analyzers and their association with disease should be further investigated. MPV can provide additional data regarding the treatment outcome in RA.

Final conclusions

1. Smokers have responded poorly to treatment with anti TNF-α biological therapy when compared to non-smokers. They have installed more quickly treatment resistance, which had led to change medication.

2. Smoking influenced the response to treatment with conventional DMARDs (smokers have changed more frequently the associated remitting medication and have used more frequently combinations of DMARDs).

3. Duration of therapy (from the initiation of biological treatment to inclusion in the present study) for the first anti TNF-α was similar between the three agents.

4. Duration of treatment for the first biological used was higher in patients with rheumatoid factor negative and non-smokers.

5. Persistence to treatment (at time of initiation of treatment until biological change) was similar in the three anti-TNF-α agents.

6. Side effects and inefficiency were the most important reasons to change the biological treatment. The most common side effects are allergic reactions, injection site skin reactions, infections and pulmonary tuberculosis. Of biological therapeutic agents, infliximab was frequently associated with adverse reactions to perfusion and TB.

7. Decrease of the score DAS28 was greater in those who switched to rituximab than those who switched to another anti-TNF-α.

8. Duration of the second and third biological agent was lower compared to the first anti-TNF-α.

9. Dosage of prednisone decreased after 1 year of treatment with TNF-α blocker from 9.6 ± 4.3 mg to 6.4 ± 4.5 mg ($p < 0.05$).

10. After 1 year of treatment with TNF-α blockers, about 10% of patients discontinued treatment with glucocorticoids, and in about half of patients reduced the dosage.

11. Smokers have used higher doses of glucocorticoids after 1 year of treatment with anti TNF-α compared with nonsmokers (8.1 ± 4 mg versus 5.8 ± 4 mg, $p < 0.05$).

12. Men have used higher doses of corticosteroids than women after 6 months and 1 year of treatment with anti TNF-α agents.

13. Biological treatment with anti TNF-α allowed dose reduction or discontinuation of corticosteroids, reducing their risk of side effects.

14. In our study, after treatment, platelet count decreased and MPV increased and was negatively correlated with platelet count.

15. MPV did not correlate with inflammatory markers or disease activity.

16. Our results complete the literature data regarding changes of MPV in conditions associated with a high degree of inflammation.

17. In our study, the conditions associated with an increased risk of thrombotic events (diabetes, dyslipidemia, hypertension) influenced platelet indices.

18. Usefulness of platelet indices, which are today analyzed by routine automated analyzers and their association with disease activity should be further investigated. MPV can provide additional data regarding the treatment outcome in RA.

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