# Anti-TNF therapy and risko of malignancies and infections in inflammatory rheumatic diseases

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**Master's thesis / Diplomski rad**

**2016**

*Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj:* **University of Zagreb, School of Medicine / Sveučilište u Zagrebu, Medicinski fakultet**

*Permanent link / Trajna poveznica:* https://urn.nsk.hr/urn:nbn:hr:105:561686

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# Anti-TNF therapy and the risk of malignancies and infections in inflammatory rheumatic diseases

**GRADUATION PAPER**

**Zagreb, 2016**

This graduation paper was made at the department of Rheumatology and Rehabilitation at Clinical Hospital Centre Zagreb under supervision of doc. dr. sc. Porin Perić and it was submitted for evaluation in the academic year 2015/2016

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# Abbreviations:

Anti-TNF – anti-tumor necrosis factor

AS – Ankylosing spondylitis

DMARDs – Disease-modifying anti-rheumatic drugs

IR – Incidence rate

IRR – Incidence rate ratio

PsA – Psoriatic arthritis

RA – Rheumatoid arthritis

RF – Rheumatoid factor

SpA - Spondyloarthropathy

TNF – Tumor necrosis factor

TNFi – Tumor necrosis factor inhibitor

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# 1. Abstract


## Objective

To evaluate the risk of infections and malignancy in a group of patients with inflammatory rheumatic diseases treated with anti-TNF therapy.

## Methods

The study involved 79 adult patients with rheumatoid arthritis (RA) and ankylosing spondylitis, psoriatic arthritis or undifferentiated spondyloarthropathy (spondyloarthopathy (SpA)); receiving anti-TNF therapy at the department of Rheumatology and Rehabilitation, Clinical Hospital Center Zagreb. The duration of therapy was a minimum of 1 month, with the mean duration of 32,0±24,0 months. The infections recorded were infections that appeared during treatment or soon after the treatment was stopped.

## Results

During the course of therapy 17 patients (21,5%) experienced an infection, with the total number of 21 infections. This resulted in an overall incidence rate (IR) of 9,9/100 patient-years. Of the patients with RA 76,5% developed an infection, which was significantly higher than for patients with SpA ($p<0,001$). The IR/100 patient-years for all infections in RA patients was 23,7 compared to 2,8 in patients with SpA. Female gender was associated with a significantly higher infection rate (70,6%, $p=0,005$). There were 8 infections that were considered serious, yielding an IR of 3,8/100 patient-years. There was only one malignancy case in our study.

## Conclusion

Every fifth patient developed an infection during the course of anti-TNF therapy, and more than one third of all infections were serious. RA and female gender was associated with a significantly increased number of infections.

# 2. Introduction

The introduction part of this paper will present the basics of the inflammatory rheumatic diseases; explain the various anti-tumor necrosis factors (anti-TNFs) that are in use, as well as, address what is known today about their association to infection and malignancy.

Rheumatic disease is an umbrella term used to describe disorders that mainly affect the joints, tendons, ligaments, muscle and bones. The characteristic symptoms are pain, stiffness and swelling of the affected areas (NIAMS 2014). Since rheumatic disease is a broad term, we are speaking of many different single disorders within this term. To narrow the disorders that are in the scope of this paper we will address the rheumatic diseases that have an inflammatory mechanism as their primary pathogenesis. This will include: rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and undifferentiated spondyloarthritis.

## 2.1 Rheumatoid arthritis

RA is defined as a chronic systemic inflammatory disorder that affects various tissues but primarily joints. It is characterized as a symmetric erosive synovitis most commonly of the peripheral joints and especially the small joints of the wrist, metacarpaophanalgeals and metatarsophalangeals. RA has a prevalence of 1% in the world's population and is most common in people aged 40-70. The disease has a higher incidence in females, with a 3:1 ration compared to males. Genetic susceptibility is a contributor to the disease and the HLA-DRB1 alleles have been associated with it. Around 80% of patients are seropositive to rheumatoid factor (RF) and the antibodies to cyclic citrullinated peptide

(CCP), the latter being more specific due to the fact that RF can be present in some people without concomitant RA. When it comes to the pathogenesis of the disease much remains uncertain, although, it is believed to be triggered by a genetically susceptible host being exposed to a certain arthritogenic antigen. This exposure is thought to render the self-tolerance to ones own cells that contain these antigens and cause an immunological attack on them. An autoimmune reaction is started and chronic destruction takes place (Kumar et al. 2010). The key players of the autoimmune reaction of RA are T-cells, B-cells and cytokines. Differentiated Th 17 cells produce IL-17, which contribute to the synovitis. B-cells produce autoantibodies and cytokines that further enhance this process. Local influx and activation of mononuclear cells, synovial fibroblasts, chondrocytes and osteaoclasts into the synovium all contribute to the destruction of the joint. The release of cytokines, especially TNF-α, IL-6 and IL-1, are important factors to the inflammation and they are also greatly responsible for some of the systemic effects in the body (Choy 2012). The pathology hallmark of the joint is hypertrophy of the synovial membrane. A pannus (activated rheumatoid synovium), containing an increased amount of the cells mentioned above, extends into and over the articular surface and ultimately destroys it by the release of metalloproteinase's and collagenases, triggered by the cytokines (Kumar et al. 2010). The systemic effects in RA are the outcome of vasculitis and lymphocytic infiltration causing the tissue damage. RA is an independent risk factor for atherosclerosis and cardiovascular diseases and persons with RA have up to three-fold increased risk for cardiovascular events and 50% increased CVD mortality (Avina-Zubieta et al. 2008, Rincon et al. 2001). A frequent finding in these people is low total cholesterol and high-density lipoprotein (HDL) and high

triglyceride lipoprotein, low-density lipoprotein (LDL) and free fatty acids (Sattar et al. 2003). There is also an increased risk of hematological malignancies, non-melanoma skin cancers and lung cancers in these individuals (Askling et al. 2005, Gridley et al. 1993).

When it comes to therapy of RA the goal is to induce remission of the disease or the lowest possible disease activity. There are various methods of assessing the disease activity and severity. The golden standard has been HAQ – Health Assessment Questionnaire developed in 1980 and still used today (Bruce & Fries 2003). Over the year there have been various modifications to HAQ and several new methods of assessing the disease have been developed. According to the new guidelines from the American Collage of Rheumatology (ACR) the use of six different measures was recommended: Clinical Disease Activity Index (CDAI), Disease Activity Score with 28-joint counts (DAS28), Patient Activity Scale (PAS), PAS-II, Routine Assessment of Patient Index Data with 3 measures (RAPID-3), and Simplified Disease Activity Index (SDAI) (Anderson et al. 2012). For the most, DAS28 has been used in this study, it shows a score from 0-10 and is calculated on the basis of the number of swollen and/or painful joints together with a more objective measure like C-reactive protein (CRP) or erythrocyte sedimentation rate. The level of RA disease activity can be interpreted as low ($DAS28 \leq 3.2$), moderate ($3.2 < DAS28 \leq 5.1$), or as high disease activity ($DAS28 \geq 5.1$) (Gestel et al. 1998).

## 2.2 Seronegative Spondyloarthropathy

This term comprises a group of diseases that share some similarities. They differ from RA in many aspects, but have similar inflammatory process of the joints. The characteristic of seronegative spondyloarthropathies (SpAs) is that they commonly affect the sacroiliac and vertebral joints, as well as, causing enthesopathies. Seronegative is used to denote that the autoantibodies connected to these disorders have not been found. Most of the SpAs are more or less associated with the HLA-B27 allele, which is believed to be a genetic factor predisposing individuals to these disorders (Reveille 1998). The individual disorders within the SpA are: Ankylosing spondylitis (AS), psoriatic arthritis (PsA), enteropahtic arthritis (EA), reactive arthritis (ReA) and undifferentiated spondyloarhtropathy (USpA). AS is the prototype, with the other diseases being more or less similar to AS. AS and PsA will be addressed here in greater detail, because they are treated in similar way to RA when it comes to anti-TNF therapy. Undifferentiated spondyloarthropathy (USpA) sometimes represent an early phase or incomplete form of AS or other spondyloarhtropathies and there is no need to address it further (Kumar et al. 2010).

### 2.2.1 Ankylosing spondylitis

AS is also known as Marie-Strumpell disease or von Bechterew. AS occurs in 0,2% of the population and is one of the inflammatory disorders that are more common in males than in females (3:1) with patients usually developing it at a younger age (Kumar et al. 2010). 90% of people with AS have the HLA-B27 allele, however, less than 5% of people that are HLA-B27 positive develop the disease (Shamji et al. 2008). The primary

pathology of the AS is caused by CD4+ and CD8+ T lymphocytes and macrophages. TNF-α and TGF-beta are two particularly important cytokines in the inflammatory process leading to fibrosis and ossification at the point where the tendon attaches to the bone (Brent 2015). The sacroiliac joints are affected in all cases and enthesitis and uveitis are also common presentations. The peripheral joints are less commonly affected, but when they are they have a different histopathology compared to RA (Kruithof et al. 2005). In clinical practice, as for RA, it is important to measure the disease activity and various scoring systems are used. BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) focuses on pain, morning stiffness and fatigue; BASMI (Bath AS Metrology Index), BASFI (Bath AS Functional Index) and BAS-G (Bath AS Patient Global Score) are other scoring indexes that are much used (NASS 2009). These measures are used to assess whether patients are eligible for treatment and if the treatment improves the disease activity.

### 2.2.2 Psoriatic arthritis

PsA develops in more than 10% of people with psoriasis and is associated with the HLA-B27 and HLA-Cw6 genes. Subsets of patients develop joint disease before the psoriatic skin lesions. Asymmetric oligoarthritis is the most common presentation and also considered to have a better outcome. With time it becomes polyarticular and these patients have an increased risk for disease progression. Severe bone and joint lysis causes shortening of digits and can lead to the development of arthritis mutilans, which is specific for PsA. Around 40% have sacroilitis, and enthesitis is common (Gladman et al. 2005). When it presents as oligoarthritis it has a similar clinical picture to RA, however, histopathological it is more similar to AS (Kruithof et al. 2005). The interplay between T

cells, monocytes and macrophages is thought to be responsible for the joint damage, like for the other inflammatory rheumatic disorders. Some cytokines are more prevalent in PsA than in RA, again showing why these disorders are different (Hammadi et al. 2015).

## 2.3 Treatment of inflammatory rheumatic arthritis

There is no cure to the inflammatory rheumatic diseases and the current approach is to arrest or slow down the progression of tissue destruction, in addition to, relive the pain and stiffness, and promote good physical motility. NSAIDs, with their anti-inflammatory and analgesic effect, are extensively used. Glucocorticoids are also appreciated for their anti-inflammatory effect and have shown to slow down new bone erosions. Another group of important agents is the disease-modifying anti-rheumatic drugs, designated DMARDs. They decrease inflammation, improve symptoms, and slow the bone destruction in RA and PsA. Their principal mechanism of action is suppression of lymphocytes and other inflammatory mediators. The newest group of drugs, for treatment of inflammatory rheumatic diseases, is designated biological DMARDs. In this group we find the anti-tumor necrosis factors (anti-TNFs) along with some other type of drugs (Katzung et al. 2012).

The positive response to anti-TNF was first established in a double-blind placebo controlled trial with infliximab, the first anti-TNF released on the market. Approximately 60% of patients receiving infliximab achieved the 20% Paulus criteria for response ($P < 0.001$ versus placebo) (Maini et al. 1998). Since then anti-TNF therapy has gained a central role in the treatment of inflammatory rheumatic diseases and some other diseases.

The newest guidelines for the treatment of RA were developed in 2015 by ACR. DMARD monotherapy, preferably methotrexate (MXT), should be commenced as soon as the disease is established. If the disease severity is moderate or severe despite the monotherapy there are further strong recommendations to add another DMARD or TNF inhibitor (TNFi) or a non-TNF biological to the therapy (Singh et al. 2015). For AS the mainstay of treatment is a full dose of NSAIDs. However, some patients do not respond, or respond poorly to this therapy. Since DMARDs did not show to be effective in AS, the advent of TNFi greatly improved the disease outcome. Today patients that do not respond to NSAIDs are advised to move directly over to anti-TNF therapy (Ward 2015). According to the European League Against Rheumatism (EULAR), for treatment of PsA, it is recommended to start with NSAIDs, if there is no sufficient response then DMARDs or local corticosteroid injection should be considered. If they fail to respond to this regime or if the disease activity is very high a TNFi is initiated (Ramiro et al 2016).

## 2.4 Tumor necrosis factor and its inhibitors

Tumor necrosis factor, cloned and characterized more than 20 years ago, was originally described as a macrophage-derived endogenous mediator that could induce hemorrhagic necrosis of solid tumors and destroy some tumor cell lines *in vitro*. Unfortunately, its promising use as an anticancer agent was limited by its toxicity as seen with the first clinical trials with TNF in the treatment of cancer. About the same time, it was found that TNF was identical to a mediator responsible for cachexia associated with cancer and sepsis, named cachectin. This research led to the conclusion that TNF is, in fact, the main lethal mediator of sepsis, as well as the publication of articles showing that TNF inhibits

the toxic effects of bacterial endotoxins, something that is now described as the systemic inflammatory response. Although clinical trials with anti-TNF in sepsis were not very successful, these studies ultimately led to the identification of TNF as a pro-inflammatory cytokine and the development of anti-TNF molecules (Sedger & McDermontt 2014). Since then, TNF-alpha has been found to play a major role in the cytokine cascade occurring in the joints of patients with RA and similar inflammatory diseases, where it stimulates the production of other inflammatory mediators and continues recruitment of immune and inflammatory cells into the joint (Scott & Kingsley 2006).

There are five anti-TNF drugs on the market today that all bind TNF-alpha: adalimumab, certolizumab pegol, etanercept, golimumab and infliximab. Adalimumab is a completely human IgG1 monocloncal antibody that blocks the interaction of TNF-alpha with TNF receptors. It also lyses cells expressing TNF-alpha in the presence of complements. Certolizumab pegol, also known as just certolizumab, is a recombinant humanized fragment antigen-binding (Fab) fragment that binds to TNF-alpha and neutralizes its activity. Etanercept, the only one of them that is a recombinant fusion protein, binds to both TNF-alpha and beta. It has similar effects to adalimumab and infliximab. Golimumab is also a humanized IgG1 monoclonal antibody, but it does not lyse cells expressing membrane-associated TNF-alpha like adalimumab (Katzung et al. 2012). Infliximab was the first of them to be approved by the U.S. Food and Drug Administration (FDA) in 1999 for the treatment of rheumatoid arthritis that did not respond to methotrexate (FDA 1999a). Infliximab is a chimeric IgG1 monoclonal antibody possessing human constant regions and murine variable regions (Sedger &

McDermontt 2014). It has same activity as adalimumab and etanercept. Despite their similar mechanism of action the individual drugs can cause a different response in different patients. Some patients that do not respond or stop responding to one of the tumor necrosis factor inhibitors (TNFis) can still respond to one of the other when switched (Carmona 2008).

## 2.5 Anti-tumor necrosis factor and infections

In the early clinical trials with infliximab it was noticed that people treated with anti-TNF therapy had higher number of infections compared to placebo. However, the safety database was regarded as small and no conclusion could be drawn. The beneficial effects of the drug were evaluated and proved to have a higher benefit then risk associated with the adverse effects. The physicians and patients where further advised to report the adverse events to assess this safety issue further (FDA 1999b). The clinical trials of etanerecept also showed some connection to risk of infection and anti-TNF use, especially upper respiratory infections (29% and 33% in the 10mg and 25mg groups respectively compared to 16% in controls) (FDA 1998). Since then, the relationship between anti-TNFs and the risk of acquiring infection has been the topic of many research papers. Opportunistic infection not common to the normal population like: Coccoidiodes immitis, Listeria spp., Histoplasma capsulatum, Aspergillus spp., Nocardia spp., mycobacteria and streptococci, have all been reported (Crum et al. 2005). The incidence of tuberculosis (TB) was the most striking and was higher than for the baseline risk of the population in some studies (Keane et al. 2001, Gomez-Reino et al. 2003). This awareness lead to new guidelines that recommends to screen for TB before commencing

anti-TNF therapy, which helped to decrease the incidence of reactivation of latent TB (Ding et al. 2010). Studies conducted have showed a small but significant increased risk of serious infections (SIs) in patients treated with TNFis (Dixon et al. 2006, Galloway et al. 2010). Serious infections were here defined as either requiring hospitalization and/or IV antibiotics or leading to death. German and Swedish Biologics Registries reported similar results (Askling et al. 2007, Listing et al. 2005). These data along with other data suggest that there is a small but significant overall risk of SI. However, other studies failed to show an increased risk (Westhovens et al. 2006, Weisman et al. 2007) and it is difficult to conclude if it is the anti-TNF or other factors like the disease it self, that predispose to more SIs. Several studies have shown that people with RA compared to the general population have an almost double increased risk of infection (Atzeni et al. 2008, Doran et al. 2002, Baum 1971). This is believed to be related to the disease it self, as well as, to concomitant use of immunosuppressant drugs. A study by Favalli et al. found an increased risk associated with age, erythrocyte sedimentation rate (ESR) and the use of steroids (Favalli et al. 2009). Other studies have also found other factors to increase the risk apart from the anti-TNF therapy, making it difficult to distinguish what is attributable only to the TNFi.

## 2.6 Tumor Necrosis Factor and Malignancy

TNF was initially found to have a tumor necrosis action in mice and accordingly named so (Carswell et al. 1975). Therefore, it was thought that when blocking its effect, with anti-TNF drugs, that it could cause the development of cancers. However, pre-clinical and clinical studies with anti-TNF therapy on humans have failed to clearly answer if it is associated with an increased risk of malignancy. A post-marketing study from Mayo

Clinic, by Bongartz et al, showed a three-fold increase in the risk of developing cancer in patients receiving infliximab or adalimumab therapy, compared to placebo (Bongartz et al. 2006). However, there was no person-year incidence rate calculated in the study and a commentary article showed the patients treated were studied for a longer time than the placebo group. When it was adjusted for time the results that Bongartz found were not statistically significant (Dixon et al. 2006). A Swedish study found that there was no overall tumor risk in treated patients, however, a proportional hazard analysis of lymphomas was done yielding a risk of 4,9 (95% CI:0,9-26,2) (Geborek et al. 2005). A subsequent analysis done later adjusted for age, gender and duration of disease did not show any significance in the risk of developing lymphoma (Askling et al. 2005). BIOBADASERs extensive study done in Spain showed no increase in rate of malignancy between exposed and non-exposed groups. Many studies have been carried out failing to provide substantial evidence. Possible explanation to this could be due to the nature of the diseases. It is known that inflammation it self is a risk factor for cancer (Coussens & Werb 2002). This means that the increased risk of cancer in patients with inflammatory diseases treated with anti-TNF could be a result of the underlying disease process and not the effect of the therapy (Simon et al. 2015). More over, RA patients treated with anti-TNF therapy are more likely to have a more severe disease activity that is associated with an increased inflammation and can be responsible for a higher number of cancers.

## 3. Methods and Statistics

This study included 79 patients treated at the department for Rheumatology and Rehabilitation at Clinical Hospital Centre Zagreb, Croatia, from 2004 to 2015. Patients were diagnosed with RA, AS, PsA or USpA. The latter three diseases were considered as one group, SpA (seronegeative spondyloarhtropathies). Data was gathered from medical files and follow-up interviews. There were four doctors involved in this study and each doctor was responsible for entering the information of their own patient. Patients were selected and treated according to guidelines from American Collage of Rheumatology (ACR) and Croatian Society for Rheumatology 2013 guidelines.

The patients included in the study received anti-TNF therapy with infliximab, adalimumab, etanercept, golimumab or cetrolizumab pegol for at least one month. The duration of the treatment was from 1 month to 109 months (9 years) with the mean duration of 32,2 months (SD±23,8). In particular, patients were excluded in the presence of any active infection after screening with the tuberculin skin test (TST), chest radiograph and hepatitis B (HBV) and C (HCV) viral markers. If patients had to pause the treatment due to side effects or infections, this time was subtracted from the main duration. Patients were also assessed for their use of DMARDs and glucocorticoids. Disease activity scores used for RA were DAS28 and HAQ, for SpA it was BASDAI and BASFI. These scores were not compared, but they were included to give the impressions of the disease activity. We used the score that was calculated before treatment.

Patients that did not respond to therapy or had severe adverse effects were taken off the therapy they were receiving and/or switched to another agent. For patients that underwent

surgery therapy would be stopped for a certain period before and after. Only infections occurring during therapy or 1 month after were noted. All types of infection, both serious and non-serious, were recorded. Infections that were defined as serious were either life-threatening, requiring hospitalization, IV antibiotic therapy and/or caused death.

Due to the fact that many patients were switched between the various anti-TNF agents it would have been difficult to estimate what agent was responsible for the infection or malignancy. Therefore, we considered the anti-TNF as one group and did not analyze the risk of each single agent.

### 3.1 Statistics

Proportions were calculated for the demographic and clinical characteristics of all patients. For important clinical characteristics, incidence rates (IRs), defined as the number of observed events (infections) or persons with infection/100 patient-years of follow-up, were calculated, to estimate the risk of infection in the different groups. T-distribution, F-variance and Pearson-Chi Square were used to calculate the probabilities. All analyses were performed using Statistica versions 7.0.

# 4. Results

## 4.1 Data of the study objects (Table 1)

Data provided in table 1 show the baseline demographics of the study group.

**Table 1:** Baseline demographics and clinical data.

| | **All patients 79** | **RA 23 (29,1%)** | **SpA 56 (70,8%)** | **p-value** |
|---|---|---|---|---|
| **Age (years)** | 46,4±11,2 | 50,5±11,8 | 44,8±10,7 | 0,04 |
| **Age beginning of therapy** | 43,9±11,8 | 46,7±13,0 | 42,8±11,2 | ns |
| **Females** | 33 (41,7%) | 20 (86,9%) | 13 (23,2%) | <0,0001 |
| **Males** | 46 (58,2%) | 3 (13,0%) | 43 (76,8%) | <0,0001 |
| **Disease duration (years)** | 11,4±8,9 | 12,6±7,2 | 10,9±9,6 | ns |
| **Therapy duration (months)** | 32,0±24,0 | 37,2±24,2 | 30,2±23,6 | ns |
| **DMARD therapy** | 35 (44,3%) | 15 (65,2%) | 20 (35,7%) | 0,016 |
| **Corticosteroid therapy** | 51 (64,6%) | 19 (82,6%) | 32 (57,1%) | 0,032 |
| **DAS28** | - | 6,2±0,9 | - | - |
| **HAQ** | - | 1,8±0,6 | - | - |
| **BASFI** | - | - | 6,58±1,73 | - |
| **BASDAI** | - | - | 6,56±1,42 | - |

DAS28 = Disease activity score; HAQ = Health Assessment Questionnaire; DMARDs = Disease-modifying anti-rheumatic drugs. Continuous variables expressed as mean values±S.D. ns: not statistically significant.

There were 56 patients with SpA (33 AS, 16 PsA and 7 USpA) and 23 with RA. The mean age of patients with RA and SpA was 50,5±11,8 and 44,8±10,7 years, respectively, showing a statistical significant difference in age between the patients of each group ($p<0,04$). In total 58% of the study candidates were male and 43% female. There were significantly more females with RA compared to SpA (Pearson Chi-square 27,2, df 1, $p<0.0001$ and t-value -2,1, df 77, $p<0,05$ respectively). Between the two groups there was no statistically significant difference in duration of disease or duration of therapy, with the total average of 11,4±8,9 years and 32,0±24,0 months respectively. In total 44,3% of all patients were receiving or had previously been receiving DMARDs, and 64,5% had been treated with oral corticosteroids. A greater percentage of subjects in the RA group were receiving DMARDs and glucocorticoids compared to SpA. For the patients with RA an average DAS28 score was 6,2±0,9 and HAQ 1,8±0,6. Average BASFI and BASDAI for the SpA group was, 6,58±1,73 and 6,56±1,42, respectively.

## 4.2 Infections (Table 2)

Baseline demographics of patients that developed an infection are depicted in table 2.

**TABLE 2:** Baseline demographics and clinical data of patients with infection.

| | Patients with infection | p value |
|---|---|---|
| Total | 17 (21,5%) | |
| RA | 13 (76,5%) | <0,001 |
| SpA | 4 (23,5%) | <0,001 |
| Age | 47,6±10,2 | ns |
| Age beginning of therapy | 43,0±11,1 | ns |
| Female | 12 (70,6%) | 0,005 |
| Male | 5 (29,4%) | 0,005 |
| Duration of disease (months) | 12,9±7,2 | ns |
| Duration of therapy | 40,1±26,7 | ns |
| DMARDs | 8 (47,0%) | ns |
| Corticosteroids | 10 (58,8%) | Ns |
| DAS28 (RA) | 6,1±1,0 | Ns |

DAS28 = Disease activity score; HAQ = Health Assessment Questionnaire; DMARDs = Disease-modifying anti-rheumatic drugs. Continuous variables expressed as mean values±SD. ns: not statistically significant

At least one infection was detected in 17 patients (21,5%) had at least one infection, with the total number of infections being 21. The total incidence rate (IR) of patients that developed an infection was 8,0 per 100 patient-year. The mean age of the patients with infections was 47,6, which did not differ much from the mean age of the patients without

an infection (47,5 years). Our study showed that two factors were associated with an increased risk of developing and infection, and this was the type of inflammatory rheumatic disease and gender. We noticed a much higher number of infections in RA patients treated with anti-TNF therapy compared to patients with SpA (13 (76,5%) vs 4 (23,5%) respectively, $p<0,001$). From our basic calculation this showed us that more than half (56,5%) of patients that suffered from RA developed an infection, compared 7,1% of patients with SpA (Risk ratio (RR) 7,9). Female gender was also greatly associated with the development of an infection ($p<0,005$). More than one third of all women developed an infection (36,4%), compared to 10,8% of males (RR 3,4).

We performed Pearson Chi-Square to assess whether there was any significance in the subjects with infection and the use of DMARDs or corticosteroids, however, no significance was shown (DMARDs Chi-square: 0,26 $p=0,61$; corticosteroids Chi-square: 0,28, $p=0,59$).

We did a t-distribution on the mean DAS28 score between subjects with RA that developed an infection compared to the subjects with RA that did not, (t-value: 0,82, df: 19, $p=0,42$), without revealing any significance.

### 4.3 Type of infection (Table 3)

Table 3 depicts the different types of infections and separates them into serious and non-serious. A total of 21 infections were detected (61.9% non-serious and 38% SIs), and three was the maximum number of infections per person. Nearly one third of all infections were serious, this is a higher than what is expected to be normal.

**Table 3: Frequency and seriousness of each infection**

| Infection | Category | Count |
|---|---|---|
| **Non-serious** | URT | 6 |
| | Bronchitis | 2 |
| | Herpes Zoster | 2 |
| | UTI | 2 |
| | Enterocolitis | 1 |
| **Total** | | 13 |
| **Serious** | Abscess in extremity | 1 |
| | Abscess in liver | 1 |
| | Necrotizing pneumonia | 1 |
| | Osteomyletitis | 1 |
| | Phlegmon extremity | 1 |
| | Pyoarthrosis | 1 |
| | Sepsis | 1 |
| | Tuberculos pleuritis | 1 |
| **Total** | | **8** |
| **All total** | | **21** |

URT=Upper Respiratory Tract, UTI=Urinary tract infection

URT were the most common type of infections the patients presented with including: sinusitis, rhinitis and throat infections. We calculated an IR/100 patient-years of 3,8 for the serious infections (SIs) and 9,0 for total number of infections. One patient died as a result of the infection, this patient developed sepsis after multiple abscesses in the abdomen.

### 4.4 Malignancy

One of the 79 patients developed a malignancy throughout the duration of the study. This shows a calculated risk if 1,25% of developing malignancy. The patient was a 55-year old male that developed hepatocellular carcinoma and died as a result of its complications. The results were regarded as not significant due to the small sample size. No further calculations were therefore carried out in relation to malignancy and anti-TNF therapy.

# 5. Discussion

We observed an IR/100 patient-years of 3,8 for the serious infections (SIs) and 9,9 for all infections in total. A study performed by Atzeni et al. showed similar results to our study (IR/1000 patient-years 31,8 equivalent to 3,2 per 100 patient-years), however, it was only conducted in patients with RA and lacked a control group (Atzeni et al. 2012).

Due to the lack of a control group in our study, we used other studies and their results as a comparison to be able to draw any conclusion as to whether anti-TNF agents are associated with an increased risk of infection. A study conducted by Salliot et al. found that the risk of SI and overall infection was 3,4 and 9,3 (IR/100 patient-years) respectively in subjects before they received any therapy with TNFis (Salliot et al. 2007). Comparing our results to their study we see that we have the same rate of infection, after anti-TNF therapy, as they had before this therapy. This would mean that if Salliot et al. findings are correct then our results do not show an increased risk of infection above what is expected in patients with inflammatory rheumatic disease. Another study conducted by Grijalva et al., from a US-multi-institutional collaboration, found the IR for SIs in the comparison group, that was not treated with TNFi, to be 7,78 and 5,37 for RA and SpA respectively (Grijalva et al. 2011). What more, this study found that the incidence rate of infections after therapy with a TNFi was 8,16 and 5,41, for RA and SpA respectively. This yielded an adjusted hazard ratio of 1,05 for both groups and was not statistically significant. We calculated our IR for SIs in RA and SpA subjects separately and the result was 8,4 (6 cases on 71,4 patient-years) and 1,4 (2 cases on 140,8 patient-years) respectively. When comparing to the group from Grijalva et al. that was not

recieving anti-TNF therapy we calculated an incidence rate ratio (IRR) of 1,07 for RA and 0.26 for SpA. This would again indicate that our study does not show any increased risk of SI in inflammatory rheumatic diseases when receiving anti-TNF therapy. To further assist in the discussion, a study found an IR of 9,6 per 100 patient-years for patients with RA not receiving TNFi, with a hazard ratio of 1,9 compared to normal matched controls (Doran et al. 2002). This study included infections requiring hospitalization in RA patients, a criteria of a SI, and since our rate for RA is lower it further strengthens the fact that we can not say that anti-TNF therapy increases the risk of infection. However, since we did not assess the different characteristics of the study candidates between our study and the other studies, it is not possible to use this discussion to draw any conclusion. Salliot et al. further found that after his subjects were treated with TNFis the IR increased (10,5 and 54,1 for SIs and all infections, respectively) and they showed an almost doubling of risk. Many other studies conducted have found an increased risk of infection with anti-TNF therapy. The German study RABBIT showed an IR of 6,4 and 6,1 for etanercept and infliximab, respectfully (Listing et al. 2005) and Dixon et al. 5,3 per 100 patient-years (Dixon et al. 2006). Our result showed just slightly lower values than in the studies above.

Our study found a significant correlation between development of infection and type of inflammatory rheumatic disorder ($p<0.0001$). Subjects with RA had a greater risk than the subjects with SpA with more than half of patients with RA developing an infection. The IR/100 patient-years for all infections in the RA and SpA group was 24,1 and 2,9 respectively (IRR 8,3). There seems to be few studies comparing the different infection risk between these two groups, however, as mentioned in the introduction RA is

associated with a higher baseline risk of infection while this risk seems to be low for SpA (Fouqué-Aubert et al. 2010). Therefore, it would be natural to expect that there were more infections in RA patients also after anti-TNF therapy. We must be careful, however, to conclude that anti-TNF therapy causes more infections in RA patients than in SpA patients. Many studies did not find any significant difference in risk of developing an infection in RA and SpA patients receiving anti-TNF (Germano et al. 2014, Grijalva et al. 2011, Salliot et al. 2007). Normally patients with SpA, especially AS, are younger and this would be abatable to bias. In our study we did have significant difference between the ages in the two groups that could be at least partially responsible for this variation.

The second factor that seemed to increase the risk of infections in patients receiving TNFis was their gender. More than one third of the females developed an infection. Female to male IRR was 2,8 (IR/100 patient-years being 12,3 and 4,4 respectively). Germano et al. showed a similar result with more than one third increased risk for females. This was also observed in other studies (Lacaille et al. 2008, Au et al. 2011). Germano et al. suspected that the reason for the increased risk was because of uro-genital tract infections (UTIs), which are known to occure more frequently in women. However, in our study there were only 2 UTIs, which could not account for the higher number of infections that was observed in females. The preponderance of women with RA compared to SpA is a probable cause of these results. We did show that there was a much higher number of infections in the RA group and 86,9% of the subjects with RA were women. Whether RA or female gender are predisposing factors to increased rate of infections is debatable, and one would need to adjust for the gender difference or have similar subject distribution in the two groups. Our study was limited when it came to that.

Since other studies also found an association between gender and infection risk in anti-TNF treated persons, we could speculate that maybe genetic factors connected to the female sex are responsible for these increased risks regardless of the type of inflammatory rheumatic disease. There should be a focus on finding out if there is any difference between infection risk and gender, if so the therapy regimes could be changed and more caution given to women that are treated with TNFis.

In the basic statistical analysis of the two groups, showed in table 1, we see that the use of DMARDs and corticosteroids in the RA group are significantly higher (p=0,016 and 0,032 respectively). When adjusted for presence of infection we did not find any statistical significance between the development of infections and the use of DMARDs or corticosteroids. However, other studies have found that concomitant use of these medications increases the risk of infection (Germano et al. 2014, Atzeni et al. 2012). The weakness of our analysis when addressing this association was that it was not known whether the patients were receiving steroids or DMARDs during the therapy with anti-TNF or if they had been receiving them sometime in the past. Possibly resulting in patients being falsely labeled as being treated with these medications during the study when in fact they were not.

The small sample size of this study (79 patients in total) makes it difficult to extract any significant data regarding infections and especially malignancies that occur at a very low rate. In addition, there is no other group to compare the results too. The ideal would be to have a cohort study with a larger sample size with one group receiving therapy and the other not, but that both groups have similar characteristics.

## 6. Conclusion

This is clearly a small sample of patients compared to other larger studies done with the same scope of interest. Compared to other similar studies the rate of SIs from our study was slightly lower, nevertheless, more than one third of the infections recorded were serious, which is believed to be high. Patients with RA and females that were treated with anti-TNFs had a significantly higher risk of overall and serious infections compared to patients with SpA and males.

## 7. Acknowledgments

I would like to thank my mentor Dr. Porin Perić for supporting me and helping me to accomplish my first scientific paper. I would also like to show my gratitude to prof. Mirjana Kujundzic-Tiljak who, with her expertise, was responsible for the statistical analysis of the data. Last but not the least I wish to thank my friends for enduring with me throughout this work and to show a special warm thank to Branimir Klobucar who helped me finalize my work.

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## 9. Biography

Ivana Sapina was born in Brcko in 1991. Because of conflicts in her country she moved to Norway at the age of 1. After graduating from high school in 2010 in Kristiansand, Norway, she directly enrolled into University of Zagreb, School of medicine. It was her wish for the past years to become a physician. She was interested in science and liked the thought of one day being able to help people with their health. Her mom had always said that one of the most important things in life is a good health. She chose to study at University of Zagreb because she always have wanted to experience more of the world, and with some of her family living in Zagreb and having a close relationship to the Croatian coast, this was not a tough decision. After six years of medical studies in Zagreb, Ivana is now ready to move back home to Norway and start her medical carrier. Her student life here in Zagreb will always stay in her heart and she intends to visit Croatia as much as she can in the future.