BRIEF PAPER

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# The effects of TNF alpha inhibition on plasma fibrinolytic balance in patients with chronic inflammatory rheumatical disorders

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**Key words:** TNF α inhibition, plasma fibrinolytic balance, PAI-1, t-PA.

## ABSTRACT

**Objectives.** *Recent studies support an inflammatory basis for atherosclerosis. Patients with chronic inflammatory rheumatical disorders are at increased risk for cardiovascular events, and this can be partially attributed to the inhibition of fibrinolytic system. TNF α inhibitors such as infliximab are shown to retard the progression of inflammatory arthritides. In this study, we investigated the effects of infliximab on plasma fibrinolytic parameters.*

**Methods.** *Thirteen patients (7 female, 6 male; mean age: 44 ± 11 years) with a clinical indication for infliximab (rheumatoid arthritis (RA) (n = 8), ankylosing spondylitis (AS) (n = 5)) were selected. Plasma plasminogen activator inhibitor (PAI-1), tissue plasminogen activator (t-PA) antigens (Ag) and high sensitive C-reactive protein (hs-CRP) levels were measured during low salt intake at baseline. All patients received infliximab (Remicaide®, i.v. infusion, 3 mg/kg). Plasma PAI-1 Ag, t-PA Ag and hs-CRP were measured during low salt intake at the end of 2 weeks. All samples were collected at 9 AM. Antigen levels were determined using a 2-site enzyme-linked immunosorbent assay.*

**Results.** *Patients experienced significant improvement in disease related activity scores after infliximab treatment. DAS score (for rheumatoid arthritis) and BASDAI index (for ankylosing spondylitis) decreased significantly after treatment (p = 0.01 and p = 0.04 respectively). Infliximab significantly reduced the marker of inflammation (hs-CRP) (8.3 ± 3.9 vs. 4 ± 4.1 mg/L, p < 0.01). Plasma PAI-1 antigen (64.7 ± 26.9 vs. 40 ± 31.1 ng/ml, p = 0.03) and PAI-1/t-PA ratio (10.8 ± 5.9 vs. 6.6 ± 3.8, p = 0.02) were significantly lower after the treatment. In contrast, plasma t-PA levels were unchanged (9.4 ± 4.4 vs. 9.0 ± 4.3 ng/ml, p = 0.73).*

**Conclusion.** *This study provides evidence that TNF α inhibition with infliximab decreases PAI-1 Ag level and PAI-1/t-PA ratio, and hence activates fibrinolytic system in patients with chronic inflammatory disorders.*

## Introduction

Recent studies support an inflammatory basis for atherosclerosis (1, 2). Patients with chronic inflammatory rheumatical disorders such as rheumatoid arthritis (RA) are at increased risk for cardiovascular events (3). The reasons for increased cardiovascular events in these patients remain unclear.

Inflammation, atherosclerosis and fibrinolytic systems share many features including involvement of cytokines such as tumor necrosis factor α (TNF α) (2, 4, 5). TNF α is shown to play a pivotal role in the initiation and amplification of the inflammatory cascade in RA (5). TNF α is also a potent agonist for PAI-1 expression and has been found to play a crucial role in PAI-1 regulation in a variety of diseases (6). Inhibition of TNF α retards progression of inflammatory arthritides such as RA and AS (7, 8). Endothelial dysfunction is common in patients with RA and anti-TNF α treatment is shown to improve endothelial function in these patients (9).

Infliximab is a chimeric monoclonal antibody directed against tumor necrosis factor α (TNF α). In this study, we investigated the effects of a TNF α inhibitor, infliximab, on plasma fibrinolytic parameters in patients with chronic inflammatory rheumatical disorders.

## Materials and methods

*Patients*

Thirteen patients (7 female, 6 male; mean age 44 ± 11 years) with a clinical indication for infliximab [RA (n = 8), AS (n = 5)] were selected. Patients were taking stable doses of methotrexate and prednisone before entering the study, however, all had high disease activity despite treatment. Disease activity was monitored with disease activity score (DAS) for patients with RA and Bath ankylosing spondylitis disease activity index (BASDAI) for patients with AS. Patients were followed up at the rheumatology clinic two weeks after the infliximab treatment. Informed consent was obtained and the study protocol was approved by the Institutional Review Board.

*Study Protocol*

Plasma PAI-1, t-PA antigens (Ag) and

**Table I.** Inflammation marker, clinical parameters and plasma fibrinolytic parameters at baseline and after infliximab treatment.

| | Baseline | After Infliximab | *P* |
|---|---|---|---|
| DAS | 5.8 ± 1.3 | 3.7 ± 2.0 | 0.01 |
| BASDAI | 5.1 ± 0.9 | 2.4 ± 1.1 | 0.04 |
| hs–CRP (mg /L) | 8.3 ± 3.9 | 4.0 ± 4.1 | < 0.01 |
| PAI–1 Ag (ng/ml) | 64.7 ± 26.9 | 40 ± 31.1 | 0.03 |
| t–PA Ag (ng/ml) | 9.4 ± 4.4 | 9.0 ± 4.3 | 0.73 |
| PAI–1/t-PA | 10.8 ± 5.9 | 6.6 ± 3.8 | 0.02 |

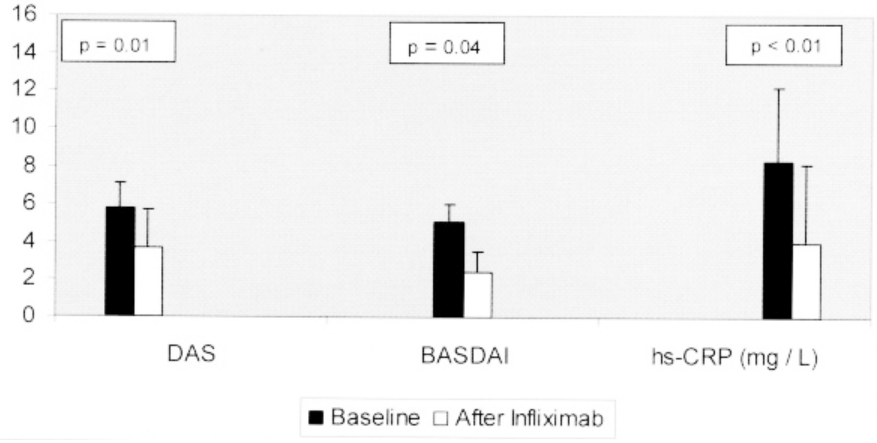


**Fig. 1.** Hs-CRP and clinical parameters at baseline and after infliximab treatment.

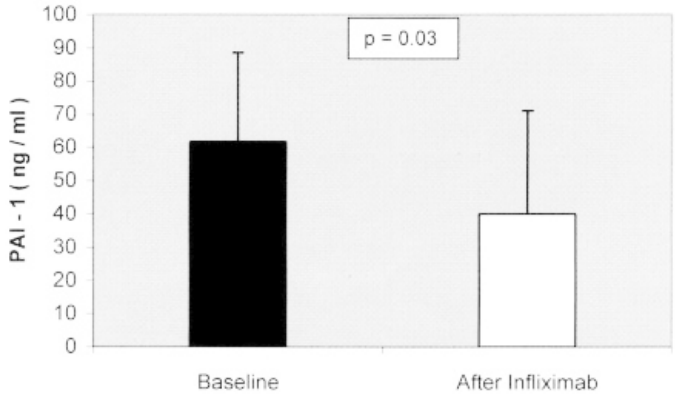


**Fig. 2.** PAI-1 antigen levels at baseline and after infliximab treatment.

high sensitive C-reactive protein (hs-CRP) levels were measured during low salt intake at baseline. Patients underwent complete history and physical examination. Disease related scores were calculated. All patients received infliximab (Remicade®, i.v. infusion, 3 mg/kg). Two weeks after the initial treatment with infliximab, patients were seen at the Rheumatology Clinic. Disease activity scores were calculated. Plasma PAI-1 Ag, t-PA Ag and hs-CRP were measured during low salt intake at the end of two weeks after treatment with infliximab. All samples were collected at 9 AM to avoid the effects of diurnal variation on plasma fibrinolytic parameters (10). Ag levels were determined using a 2-site enzyme-linked immunosorbent assay (Diagnostica Stago). The PAI-1 and t-PA mass ratio was determined by dividing plasma concentrations (ng/ml) by the molecular weights of the 2 proteins (70 kD for t-PA and 50 kD for PAI-1).

*Statistical analysis*

Results are presented as mean ± SEM. Differences in measurements before and after infliximab treatment were compared by Wilcoxon Signed Ranks Test. Non-parametric tests are used to study the correlation between disease activity scores, inflammatory marker (hs-CRP) and fibrinolytic parameters (t-PA and PAI-1). Statistical significance was accepted at $p < 0.05$.

## Results

*Inflammation markers and clinical parameters*

There was a positive and significant correlation between hs-CRP and disease related activity scores at baseline ($p < 0.01$). Patients experienced significant improvement in disease related activity scores after infliximab treatment (Table I). Disease Activity Score (DAS) was lowered from 5.8 ± 1.3 to 3.7 ± 2 ($p = 0.01$) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) decreased significantly from 5.1 ± 0.9 to 2.4 ± 1.1 ($p = 0.04$) after the treatment. Infliximab also significantly reduced the marker of inflammation (hs-CRP) (8.3 ± 3.9 vs. 4 ± 4.1 mg/L, $p < 0.01$) (Fig. 1).

*Plasma fibrinolytic parameters*

Plasma PAI-1 antigen (64.7 ± 26.9 vs. 40 ± 31.1 ng/ml, $p = 0.03$) and PAI-1/t-PA ratio (10.8 ± 5.9 vs. 6.6 ± 3.8, $p = 0.02$) were significantly reduced after the treatment (Fig. 2). In contrast plasma t-PA level was unchanged (9.4 ± 4.4 vs. 9.0 ± 4.3 ng/ml, $p = 0.73$) (Fig. 3). We did not observe any significant correlation between hs-CRP level, disease activity scores and plasma fibrinolytic parameters.

## Discussion

Patients with chronic inflammatory rheumatical disorders such as RA are at increased risk for thrombotic events (3). A recent study reported that the hospitalized myocardial infarctions (MI) were > 3 times, unrecognized MI were > 5 times and sudden death was > 2 times more prevalent in RA patients compared to the control group (3). The mechanisms through which patients with chronic inflammatory rheumatical disorders are subject to higher risk of thrombotic events remain unclear.

Endothelial function and fibrinolytic system play a central role in atherosclerosis (4).

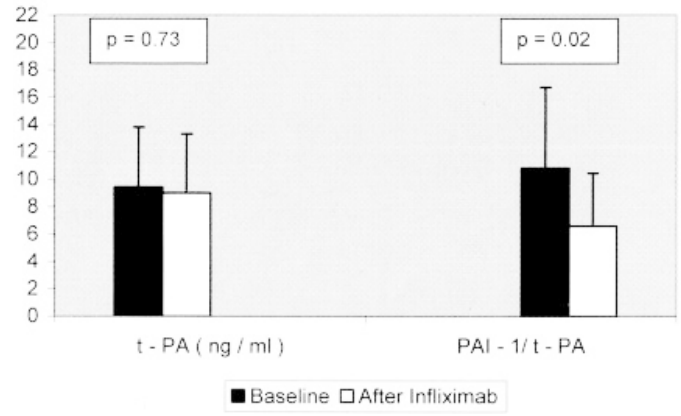


**Fig. 3.** t-PA antigen levels and PAI-1/t-PA ratio at baseline and after infliximab treatment.

The total fibrinolytic potential of human blood is determined by the interface between the plasminogen activators (t-PA) and plasminogen activator inhibitors (PAI-1). Acute cardiovascular effects of elevated plasma PAI-1 support its role as a novel cardiovascular risk factor. Clinical and experimental evidence suggest that long-term effects of vessel wall PAI-1 is crucial in vascular disease (4). Therefore, increased risk for cardiovascular events in chronic inflammatory rheumatical disorders can be partially attributed to the inhibition of fibrinolytic system and endothelial dysfunction.

We observed that TNF α inhibition with infliximab decreased PAI-1 Ag level and PAI-1/t-PA ratio, and hence resulted in activation of fibrinolytic system in patients with chronic inflammatory disorders. Our results support previous reports. Transcriptional control of PAI-1 is a pivotal mechanism in determining tissue and plasma PAI-1 content. TNF α activates human PAI-1 gene through a distal nuclear factor kappa B site in human endothelial cells (6, 11).

The fibrinolytic system represents a crucial defense mechanism against intravascular thrombosis. Activation of plasminogen is the key event of fibrinolysis (12). The initiation of fibrinolysis is regulated by a dynamic balance between t-PA and PAI-1. An imbalance can lead to vascular events. PAI-1 deficiency or t-PA excess can result in abnormal bleeding (13).

Conversely, an elevated PAI-1 level is associated with reduced fibrinolysis and increased the cardiovascular risk (14). Young myocardial infarction (MI) survivors had lower t-PA and higher PAI-1 levels compared to control subjects (14).

Endothelial cells are major sources of plasma t-PA and PAI-1 (15). The standard risk factors for endothelial dysfunction and vascular disease are hypertension, diabetes, renin-angiotensin system activation, hypertryglyceridemia, insulin resistance and post-menopausal state in women and these conditions are associated with elevated PAI-1 levels (16, 17). Endothelial function is impaired in RA patients (9), therefore an impaired fibrinolytic system can partially explain the increased prevalence of thrombotic cardiovascular events in these patients (3). In a small group of patients with RA, anti-TNF α treatment with infliximab was shown to improve endothelial function (9). The observed reductions in PAI-1 level and PAI-1/t-PA ratio with anti-TNF α treatment are likely secondary to an improvement in endothelial function.

Studies involving PAI-1 kinetics in healthy population show a molar excess of active PAI-1 over tPA (18). PAI-1/t-PA ratio in our group of patients with RA and AS appeared to be higher than the previously reported ratio in normal populations (19, 20) suggesting that fibrinolytic system is inhibited in these patients with chronic inflammatory rheumatical disorders. Also it was shown that the circulating levels of TNF α are associated with the response to infliximab and could help to identify patients who would benefit from anti TNF α treatment (21, 22).

We observed a significant improvement in the clinical and laboratory markers of inflammation after the first dose of infliximab. Previous controlled trials with the TNF α blockade in patients with resistant RA and AS also report a similar impressive improvement as early as 2 weeks (23, 24).

TNF α initiates and amplifies the inflammatory cascade (5). Inflammation marker and disease activity scores were reduced with anti-TNF α treatment in our patients. Previous studies suggest that hsCRP as a systemic inflammatory marker correlates with fibrinolytic parameters in patients with stable coronary artery disease (25). It is also possible that PAI-1 is an acute phase reactant (26). Therefore, reduced inflammation can contribute to alteration in the fibrinolytic balance after the treatment with TNF α inhibitors.

Similarly, CRP may serve as an atherothrombotic agent that impairs fibrinolysis by promoting the synthesis of PAI-1 (27). CRP directly induces PAI-1 m RNA, PAI-1 Ag and activity in human aortic endothelial cells (27). Hence, reduced inflammation and CRP levels with infliximab treatment may be the explanation for reduced PAI-1.

## Conclusion

This study provides evidence that TNF α inhibition with infliximab decreases PAI-1 Ag level and PAI-1/t-PA ratio, and hence activates fibrinolytic system in patients with chronic inflammatory disorders. Larger studies are needed to understand exactly the mechanism of action of the new therapeutic targets such as TNF α inhibition in chronic inflammatory conditions.

## Limitations

Previous studies revealed that ACE inhibition lowered PAI-1 antigen levels at the end of two weeks in normotensive subjects (28). Therefore, we measured tPA/PAI-1 Ag ratio two weeks after the infliximab treatment. However, we do not know the long term effects of infliximab on fibrinolytic parameters.

We compared tPA and PAI- 1 antigen levels before and after infliximab treatment, but we did not include a subgroup of patients treated without infliximab. However, since infliximab was the only alteration in their treatment during the 2 weeks, we suggest that

change in fibrinolytic parameters might be due to TNF α inhibition.

Previous studies report that baseline TNF α levels can predict the clinical response to infliximab (21). Unfortunately, we did not measure TNF α levels in these patients which could predict the change in fibrinolytic parameters.

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