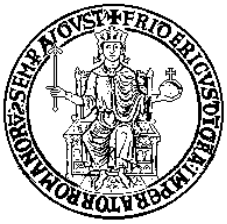

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PRIMARY AND SECONDARY HAEMOSTATIC PARAMETERS AND SURROGATE MARKERS OF ATHEROSCLEROSIS IN PATIENTS WITH PSORIATIC ARTHRITIS: FROM EPIDEMIOLOGICAL EVIDENCE TO EXPERIMENTAL STUDIES.

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INTRODUCTION

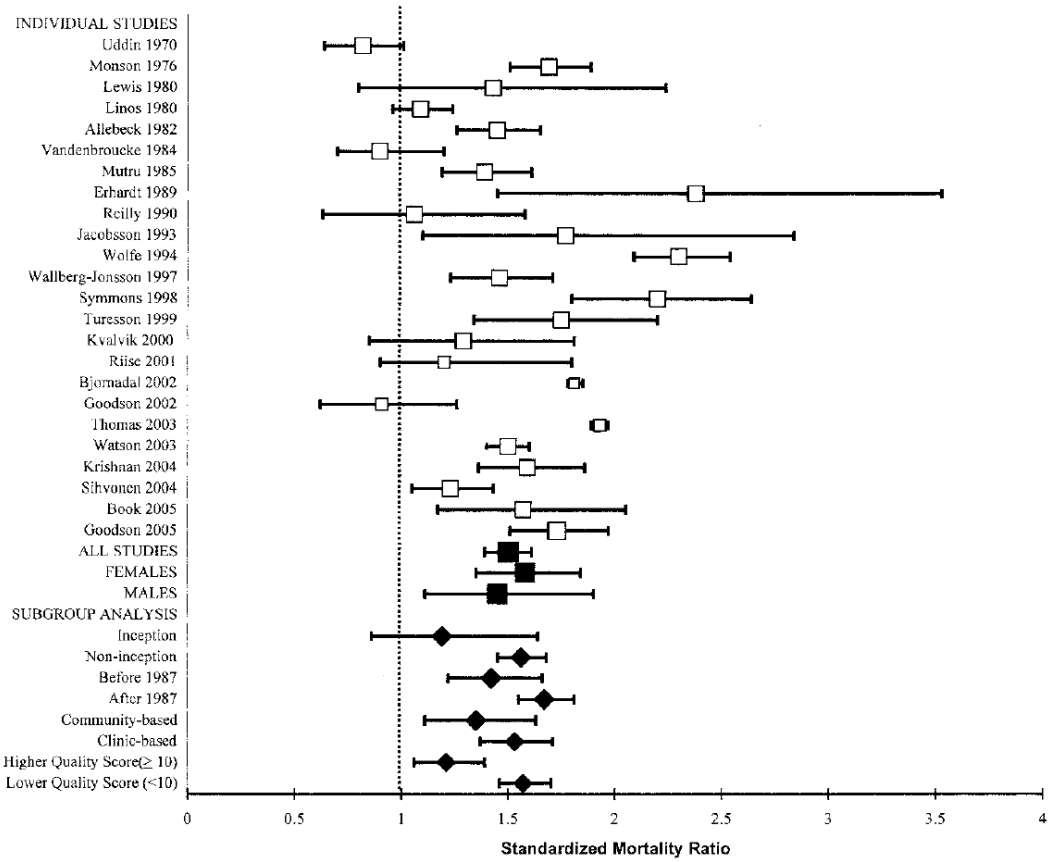
Together with the progressive disability secondary to the joint impairment, cardiovascular (CV) risk is a major issue in patients with rheumatic diseases, such as Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA).[1] In this clinical settings, a high prevalence of the metabolic syndrome and of its major features (obesity, hypertension, impaired fasting glucose, hypercholesterolemia, hypertriglyceridemia) has been described.[2] However, such an association does not entirely explain the extent of premature atherosclerosis in rheumatic subjects and inflammation appears to act synergistically with traditional vascular risk factors (VRFs), thus contributing to the atherosclerotic process and to the increased CV risk.[3,4] Monocytes, CD4+ T-lymphocytes and most pro-inflammatory cytokines (Tumor Necrosis Factor-α [TNF-α], interleukin [IL]-1β, IL-6 and IL-18), play a central role in the pathophysiology of major arthritides,[5] and are involved in the induction and in the maintenance of the atherosclerotic process (hyperlipidemia, insulin-resistance, adhesion of white cells to endothelium, over-expression of some molecules, "fatty streaks" formation and atherosclerotic plaque progression and rupture).[6,7,8] On the other hand, VRFs may play a role in the development of inflammation. For instance, being associated with the production of "adipokines" (TNF-*α*, IL-6, leptin, adiponectin), obesity causes a pro-inflammatory status.[9,10,11]

In the last years a growing interest has been devoted to the evaluation of the complex interplay among these mechanisms, to better address the issue of CV mortality and morbidity in rheumatic patients. Most of available studies have been conducted on patients with RA, a systemic, autoimmune disorder

characterized by synovial inflammation of multiple joints and by an higher than normal prevalence of co-morbidities.[12]

As to CV morbidity and mortality in RA, according to a meta-analysis of 24 observational studies (111,758 RA patients, 22,927 CV events), mortality risks for ischemic heart disease and cerebro-vascular accidents were respectively 59% and 52% higher in RA subjects than in the general population (**Figure 1**).[13] In line with these data, in a prospective study, the 3-year incidence rate of fatal and non-fatal CV events was 9.0% in RA patients and 4.3% in the general population. In addition, a recent study showed that non-diabetic RA patients and non-RA type-2 diabetics have a similar high risk of CV events as compared with healthy subjects (Hazard Ratios, HRs: 2.16, p=0.004 and 2.04, p=0.019, respectively).[14] RA subjects also exhibited a higher than normal recurrence of CV events than control subjects (57.5% vs 30%, p=0.013). Interestingly, while chest pain at presentation was referred by all controls, it was found in only 82% RA patients (p=0.006).[15] Accordingly, RA patients were twice as likely to experience unrecognized myocardial infarction (HR: 2.13, 95%CI:1.13-4.03) and sudden death (HR: 1.94, 95%CI: 1.06-3.55) as controls.[16] In keeping with these findings, post-mortem evaluations of the coronary arteries of RA patients, showed histological evidence of a higher than normal inflammation and of plaque instability as compared with controls.[17]

Figure 1. Meta-analysis of 24 studies on cardiovascular disease mortality in patients with rheumatoid arthritis.



Adapted from Aviña-Zubieta JA et al. Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. Arthritis Rheum. 2008 Dec 15;59(12):1690-7.

When evaluating CV risk factors, the metabolic syndrome was found in 40-45% of AR patients[18], with dyslipidemia (defined as an abnormal ratio in the lipid particles leading to an unfavorable atherogenic index), being present in up to 50% of patients.[19,20] Overall, compared with controls, patients with active RA show an abnormally high prevalence of several CV risk markers.[21] Carotid intima-media thickness (IMT) assessment is a non-invasive imaging test for subclinical atherosclerosis,[22,23] and it has been widely accepted as one of the strongest predictors of major CV events (stroke, myocardial infarction, heart failure, or CV death).[24,25] The presence of carotid plaques is considered an even more reliable predictor of CV events than IMT. [26] Thus,

these surrogate markers of subclinical atherosclerosis provide important prognostic information over and above traditional CV risk factors.

In a recent meta-analysis[27] we have evaluated the impact of RA on common carotid artery intima-media thickness (CCA-IMT) and on the prevalence of carotid plaques. A total of 59 studies (4,317 RA patients and 3,606 controls) were included in the final analysis, 51 studies with data on CCA-IMT (52 data-sets on 3,600 RA patients and 3,020 controls) and 35 studies reporting on the prevalence of carotid plaques (2,859 RA patients and 2,303 controls). As compared to controls, RA patients showed a higher CCA-IMT (mean difference [MD]: 0.10 mm; 95%CI: 0.07, 0.12; P <0.00001), and an increased prevalence of carotid plaques (Odds Ratio [OR]: 3.61; 95%CI: 2.65, 4.93; P<0.001). Interestingly, when analyzing studies on early-RA, the difference in CCA-IMT among RA patients and controls was even higher (MD: 0.21 mm; 95%CI: 0.06, 0.35; P=0.006), and difference in the prevalence of carotid plaques was entirely confirmed (OR: 3.57; 95%CI: 1.69, 7.51; P=0.0008).

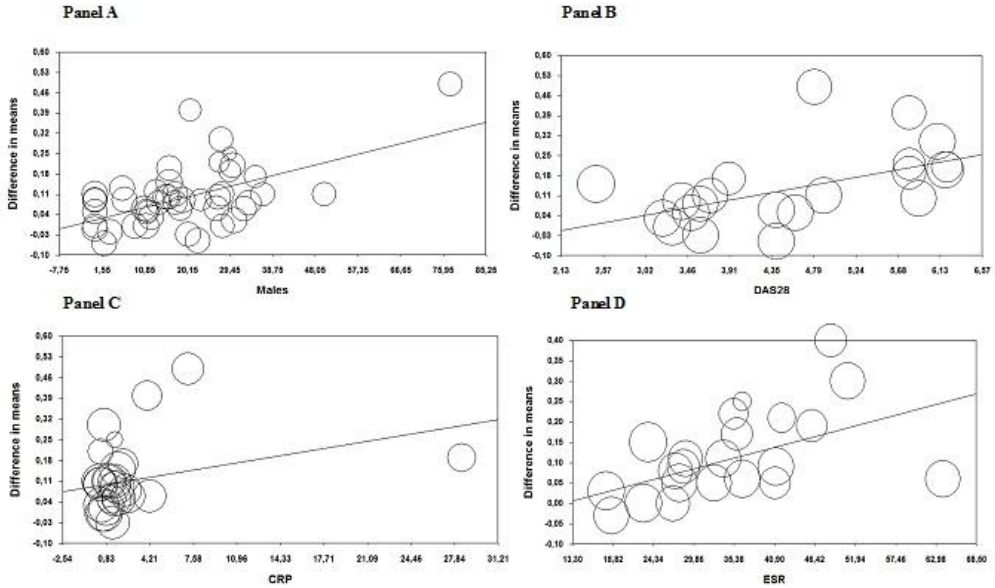
Moreover, meta-regression models **(Figure 2)** showed that male gender (Z=4.38; P=0.00001) and a more severe inflammatory status (as expressed by DAS-28, CRP levels, and ESR) significantly impacted on CCA-IMT (Z=2.95; P=0.003; Z=2.19; P=0.02 and Z=2.88; P=0.003, respectively).

In addition, we have recently demonstrated an impaired endothelial function - as expressed by flow-mediated dilation and nitrate-mediated dilation - in patients with RA[*manuscript uder review*]. In a total of 20 studies (852 RA patients, 836 controls), RA patients showed a significantly lower FMD (MD: -2.16%; 95%CI: -3.33, -0.98; P=0.0003), with no differences in NMD (MD: -0.41%; 95%CI: -2.89, 2.06; P=0.74). Interestingly, a lower FMD (MD: -2.00%;

95%CI: -3.20, -0.80; P=0.001) and no differences in NMD (p=0.49) were confirmed when excluding data on patients with early-RA. Meta-regression models showed that no clinical or demographic variable influenced the association between RA and FMD.

As to arterial stiffness, some data already suggest that RA patients show an arterial stiffness similar to patients with smoking habit, hypertension or diabetes.[28,29]

Figure 2. Meta-regression analyses. Effects of male gender (Panel A), DAS28 (Panel B), CRP (Panel C), ESR (Panel D) on the common carotid artery intima-media thickness (IMT) in patients with rheumatoid arthritis (RA).



DAS-28: disease activity score in 28 joints. CRP: C-reactive protein. ESR: erythrocyte sedimentation rate.

THE CARDIOVASCULAR RISK IN PATIENTS WITH PSORIATIC ARTHRITIS

Psoriatic arthritis (PsA) is a chronic inflammatory arthropathy affecting up to 40% of patients with psoriasis and leading to severe physical limitations and disability[30]. With a prevalence of 0.3-1% in the general population, PsA is one of the most common chronic inflammatory conditions[31]. Beyond skin and joint involvement, PsA is characterized by a high prevalence of comorbidities, such as autoimmune, infectious, and neoplastic diseases [32,33,34]. Moreover, metabolic syndrome (MetS) and its major features (obesity, hypertension, impaired fasting glucose, hyperlipidemia) have been frequently found in PsA patients,[35] and a shortened life expectancy was reported in this clinical setting, with CV disease accounting for up to 36% of the overall mortality.[36] Growing evidence on CV morbidity and mortality in PsA patients (**Table 1**) suggests that a 62% increased mortality has been reported in PsA subjects compared with the general population. An increased standardized prevalence ratios (SPR) for myocardial infarction (2.57; 95% CI: 1.73-3.80) and for angina (1.97; 95%CI: 1.24-3.12]) but not for stroke and heart failure has been prospectively reported in 648 PsA patients compared with nearly 40,000 controls.[37] More recently, Jamnitski et al[38] showed that the prevalence of CVD was 10% in patients with PsA compared with 12% in patients with RA (p=0.264), suggesting that the CV risk profile of PsA subjects resembles that of RA. Two reports[39,40] failed to show an increased risk of death in PsA patients as compared with healthy controls. As both were community-based rather than clinic-based studies, this finding may suggest a relationship between disease severity and mortality, as patients with the most severe disease activity are seen at specialized referral centers.

Table 1. Studies on cardiovascular morbidity and mortality in patients with psoriatic arthritis.

Author	Study design	Controls	PsA patients	Overall risk	Adjusted risk
Myocardial infarction					
Gladman	Prospective cohort Outpatients	General population	648	SPR: 2.57	-
Li	Prospective/retrospective cohort Outpatients	93,545	2463	HR: 7.4	HR: 4.18
Coronary artery disease					
Han	Cross-sectional Inpatients/outpatients	12,264	3066	PREVALENCE: 1.3	-
Gladman	Prospective cohort Outpatients	General population	648	SPR: 1.97	-
Sommer	Retrospective case-control Inpatients	1,044	581	PREVALENCE: 3.6% vs 5.5%	OR: 1.51
Stroke					
Han	Cross-sectional Outpatients	12,264	3066	PREVALENCE: 1.3	-
Gladman	Prospective cohort Outpatients	General population	648	SPR: 0.91	-
Li	Prospective/retrospective cohort Outpatients	93,545	2463	HR: 3.18	HR: 2.40
Cardiovascular events					
Alhehoff	Prospective cohort Inpatients/outpatients	4,003,265	607	-	RR: 1.79
Jamnitsky	Cross-sectional	353 RA	489	OR: 0.79	-
Peripheral arterial disease					
Han	Cross-sectional Outpatients	12,264	3066	PREVALENCE: 1.6	-
Cardiovascular mortality					
Wong	Prospective cohort Outpatients	General population	428	SMR: 1.33	-
Buckley	Retrospective cohort	General population	453	SMR: 0.81	-
Alhehoff	Prospective cohort Inpatients/outpatients	4,003,265	607	-	RR: 1.84
Mok	Cross-sectional Inpatients/outpatients	General population	778	-	SMR: 1.59

HR, hazard ratio; 95% CI, 95% confidence interval; OR, odds ratio; PsA, psoriatic arthritis; RR, relative risk; SPR: standardized prevalence ratio

Adapted from Horreau C et al. Cardiovascular morbidity and mortality in psoriasis and psoriatic arthritis: a systematic literature review. J Eur Acad Dermatol Venereol. 2013 Aug;27 Suppl 3:12-29.

By assessing cardiovascular risk factors, PsA patients exhibit an increased prevalence of MetS as compared to both subjects with AR or with AS, (38% vs 20% and vs 11%, respectively).[41] Similarly, an increased prevalence of hypertension, hyperlipidemia, obesity and type II diabetes (Odds Ratios ranging from 1.54 to 2.59) has been found in PsA subjects as compared with those with psoriasis.[42] As to additional VRFs, hyperhomocysteinemia may be caused by medications (methotrexate and salazopyrine) often used in the treatment of arthritides[43,44] as much as by genetic and/or nutritional defects. Although many literature data support the possibility of an increased CV risk in PsA patients, the presence of traditional CV risk factors does not entirely explain the CV morbidity and mortality found in this clinical setting, inflammation appearing to act synergistically with traditional CV risk factors, thus contributing to the atherosclerotic process and to the increased CV risk. Furthermore, scores currently used for the prediction of CV risk in the general population (e.g. the Framingham score; the Systematic Coronary Risk Evaluation model),[45,46] do not take into account the potential role of inflammation.[47] Based on standard algorithms, the European League Against Rheumatism (EULAR)[1] suggested the application of a 1.5 multiplier to the risk calculated in rheumatic patients. While appealing for its simplicity, this approach requires a long-term validation in which repeated CV risk assessments in rheumatologic settings are mandatory.[1] In the meanwhile, EULAR suggested the need of studies evaluating the intimate mechanisms of the interaction between inflammation and the CV risk.

RESEARCH PROPOSAL

The primary aim of this project was to evaluate primary and secondary haemostatic parameters, as well as surrogate markers of atherosclerosis in PsA patients to understand underlying mechanisms leading to the increased CV risk in this clinical setting. To this end, and to further support the cooperation among experts in Rheumatology, Internal Medicine and Cardiology, we founded the CaRRDs (Cardiovascular Risk in Rheumatic Diseases) study group.

HAEMOSTATIC AND FIBRINOLYTIC PARAMETERS

Primary haemostasis (platelet activation/aggregation)

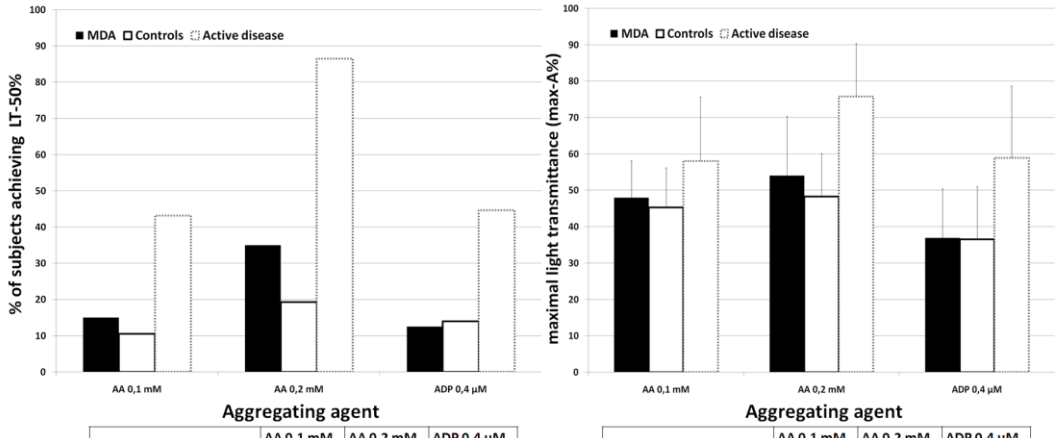
Platelet hyper-reactivity is a major predictor of arterial thrombosis and, in turn, of CV events.[48,49] Platelets produce inflammatory mediators (i.e. CD40L, myeloid-related proteins, and platelet-derived growth factor) and mediate leukocyte incorporation into plaques through platelet-mediated leukocyte adhesion. On the other hand, several cytokines/chemokines involved in PsA, by interacting with specific platelet receptors, cause intracellular calcium mobilization, nucleotide secretion and platelet activation.[50,51] These data suggest a synergism between inflammation and atherothrombosis.[52] However, little is known about the association of disease activity and platelet reactivity in PsA subjects receiving TNF-α blockers. In our study,[53] among those referred to the Psoriatic Arthritis Clinic of the Federico II University of Naples, we enrolled 114 consecutive individuals with PsA (according to CASPAR criteria[54]) receiving TNF-α blockers for ≥1 year. Healthy individuals (n=114) matched for age, gender and cardiovascular risk factors[55] served as controls. The achievement of a sustained (>1 year) minimal disease activity (MDA, according to Coates criteria[56]) was evaluated in each PsA subject. Platelet aggregability was evaluated by the assessment of the maximal light transmittance (max-A%) achieved within 5 min after the addition of very low concentrations of pro-aggregating agents (0.1 and 0.2 mM arachidonic acid [AA] or of 0.4 μM of adenosine diphosphate [ADP]).[57] The achievement of an irreversible light transmittance ≥50% (LT-50%) defined the platelet hyper-reactivity.[58] Major characteristics of case and control subjects are reported in **Table 2**.

Table 2. baseline clinical and demographic characteristics of the study population.

Variable	PsA Subjects, n = 114	Controls, n = 114	p
Age, yrs	51.48 ± 12.58	49.36 ± 10.28	0.488
Male, n (%)	56 (49.1)	43 (37.7)	0.109
Metabolic syndrome, n (%)	38 (33.3)	33 (28.9)	0.567
IFG	11 (9.6)	10 (8.8)	1.000
Hypertension	46 (40.4)	40 (35.1)	0.495
Hypercholesterolemia with low HDL	59 (51.8)	63 (55.3)	0.690
Hypertriglyceridemia	30 (26.3)	29 (25.4)	1.000
Obesity	71 (62.3)	74 (64.9)	0.783
Smoking habit, n (%)	3 (28.9)	38 (33.3)	0.567
Disease duration, mo	98.11 ± 54.12	—	—
Treatment duration, mo	48.11 ± 16.41	—	—
Pattern of PsA, n (%)			
Distal joint disease	0 (0)	—	—
Oligoarthritis	26 (22.8)	—	—
Spondylitis	40 (35.1)	—	—
Polyarthritis	42 (36.8)	—	—
Mutilans	6 (5.3)	—	—

No PsA subject was receiving traditional DMARDs. Adalimumab was employed in 44 (35.6%) subjects; Etanercept and Infliximab in 35 (30.7%) each. PsA subjects achieved more often LT-50% than controls following exposure to 0.4 μM ADP (33.3% vs 14.0%, p=0.001), 0.1 mM AA (33.3% vs 10.5%, p<0.001) or 0.2 mM AA (68.4% vs 19.3%, p<0.001). In addition, max-A% was higher in PsA subjects than in controls (AA 0.1 mM: 54.50±16.10 vs 45.28±10.93, p<0.001; AA 0.2mM: 68.14±18.45 vs 48.32±11.79, p<0.001; ADP 0.4μM: 51.15±20.68 vs 36.49±14.49, p<0.001). The rate of LT-50% achievement in the 40 PsA subjects in MDA was comparable to that of controls and significantly lower than that of subjects with an active disease (**Figure 3, left side**). Likewise, max-A% of those achieving MDA was comparable to controls and lower than that of individuals with active disease (**Figure 3, right side**).

Figure 3. Platelet aggregation in response to different aggregating agents in PsA. Relation to disease activity



	AA 0.1 mM	AA 0.2 mM	ADP 0.4 µM
MDA vs Controls	p=0.567	p=0.052	p=1.000
MDA vs Active disease	p=0.003	p=<0.001	p=<0.001
Active disease vs Controls	p=<0.001	p=<0.001	p=<0.001

	AA 0.1 mM	AA 0.2 mM	ADP 0.4 µM
MDA vs Controls	p=0.809	p=0.071	p=1.000
MDA vs Active disease	p=0.001	p=<0.001	p=<0.001
Active disease vs Controls	p=<0.001	p=<0.001	p=<0.001

AA: arachidonic acid; ADP: Adenosine diphosphate; LT-50%: irreversible light transmittance ≥50%; MDA: minimal disease activity

CRP showed a direct correlation with max-A% (AA 0.1mM: r=0.360, p<0.001; AA 0.2mM: r=0.278, p=0.003; ADP 0.4µM: r=0.224, p=0.018). Platelet hyper-reactivity is a major predictor of CV events and of arterial thrombosis[48,49] and all our findings strongly support a synergism between inflammation and pathobiology of atherothrombosis.[52] Platelet reactivity is increased in PsA patient, especially in those with an active disease. In particular, lower concentrations of pro-aggregating agents were needed by PsA subjects to achieve LT-50% than controls and the max-A% was higher in PsA subjects than in controls. However, by stratifying for the disease activity, we found that LT-50% and max-A% of PsA subjects in MDA were comparable to controls and lower than PsA subjects with active disease. These findings confirm and extend the association between inflammation and platelet reactivity. The final confirmation of such an association derives by the evidence that CRP levels directly correlated with max-A%.

Secondary haemostasis (coagulation and fibrinolysis)

Growing evidence suggests a relevant role for changes in haemostatic system parameters in the determinism of the CV risk in rheumatic patients.[59] In addition to primary hemostasis (platelet reactivity), changes in fibrinolytic (t-PA, PAI-1) and secondary hemostasis variables (coagulation proteins; natural anticoagulants) are known to play a relevant role in the CVD risk. Impaired fibrinolysis and/or raised levels of coagulation factors and/or reduced levels of natural anticoagulants (protein C, protein S, Antithrombin) have been recognized as major determinants of both arterial and venous thrombosis.[60] By enhancing platelet reactivity and affecting a series of coagulation and fibrinolytic variables, pro-inflammatory cytokines (i.e. tumor necrosis factor-a [TNFa] and interleukin 6 [IL-6]), may trigger the thrombotic risk in rheumatic patients.[53,61]

Lacking specific data on patients with PsA, we have prospectively evaluated changes in hemostatic and fibrinolytic variables in PsA subjects starting a treatment with TNF-α inhibitors.[62] In addition, we have compared changes in these variables with those found in subjects that had achieved minimal disease activity (MDA) with traditional Disease Modifying Anti-Rheumatic Drugs (DMARDs) and are on continuous treatment with such drugs.

Among PsA patients screened for inclusion in the study, 76 consecutive subjects (40 females, 36 males; mean age: 45.7±12.3 years) that were non-responders to the treatment with traditional DMARDs and eligible to start a treatment with TNF-α blockers (according to ASAS recommendations[63]) were enrolled as case group (Group 1).

In parallel, among those that had achieved MDA while on treatment with DMARDs and that were still successfully treated with such drugs at the time of the evaluation, 80 subjects (48 females, 32 males; mean age: 46.6±11.7 years), matched for age and sex with those of Group 1, were enrolled in this study to serve as control group (Group 2). In all patients, fibrinolytic (plasminogen activator inhibitor-1 [PAI-1], tissue plasminogen activator [t-PA], PAI-1/t-PA ratio) and haemostatic (Fibrinogen, D-Dimer, coagulation factors VII, VIII and von Willebrand Factor [vWF] Protein C, Protein S and Antithrombin[AT]) variables were determined at enrolment (T0) and after a 6-month follow-up (T1). Results were stratified according to treatment and to the disease activity (MDA or non-MDA).

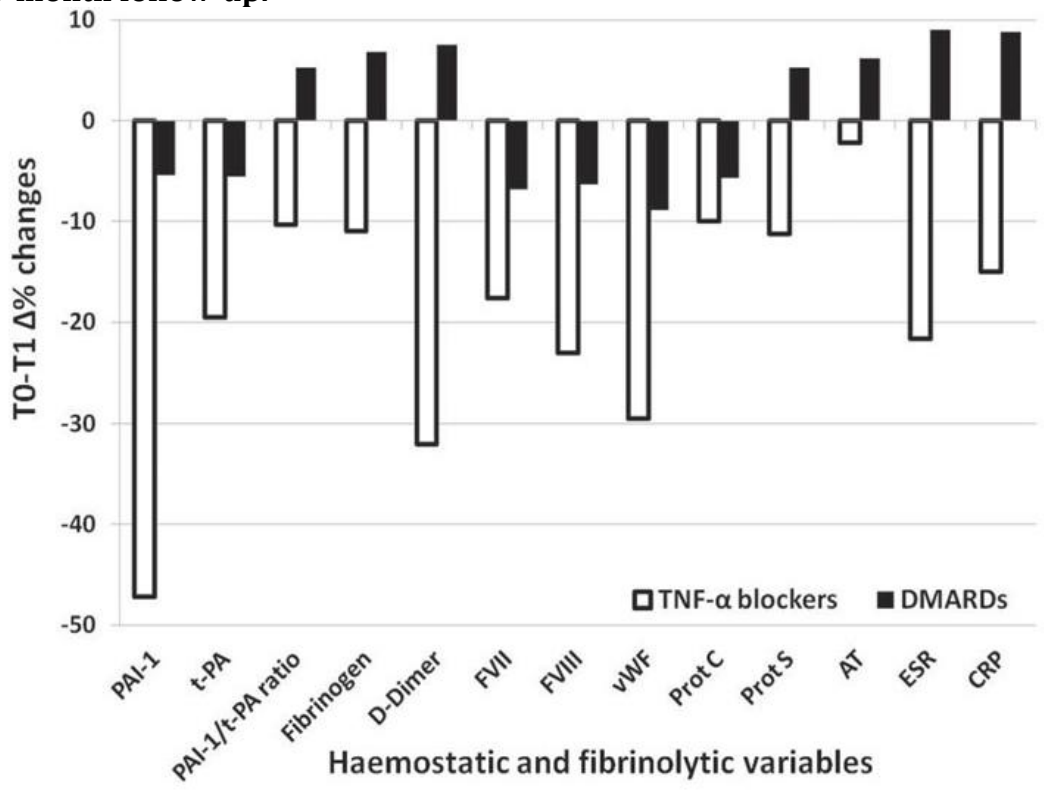
Baseline clinical and demographic characteristic of Group 1 and Group 2 subjects are reported in **Table 3**. Most of the analyzed variables were significantly lower in Group 2 (MDA) than in Group 1 (non-MDA) subjects.

Table 3. baseline clinical and demographic characteristics of the study population.

Variable	Group 1 (no-MDA), n = 76	Group 2 (MDA), n = 80	p
Age, yrs	45.73 ± 12.35	46.65 ± 11.57	0.634
Male sex, n (%)	36 (47.4)	32 (40.0)	0.420
PAI-1 antigen	67.28 ± 13.94	58.62 ± 11.14	0.032
t-PA levels	9.45 ± 2.22	8.07 ± 1.61	0.027
PAI-1/t-PA ratio	7.11 ± 5.56	7.26 ± 4.87	1.000
Fibrinogen	328.57 ± 83.05	302.12 ± 43.19	0.031
D-dimer	252.12 ± 252.12	190.47 ± 73.19	0.045
FVII	123.92 ± 20.43	107.70 ± 12.18	< 0.001
FVIII	129.01 ± 25.24	119.33 ± 17.42	0.008
vWF	138.11 ± 28.41	128.98 ± 19.96	0.029
Prot C	121.47 ± 18.25	114.25 ± 14.81	0.039
Prot S	114.57 ± 23.12	106.11 ± 15.29	0.013
AT	100.43 ± 7.62	96.36 ± 5.96	0.006
ESR	22.40 ± 13.46	18.17 ± 8.98	0.032
CRP	5.02 ± 5.83	3.46 ± 2.20	0.034

During the 6-month treatment period with TNF-α blockers, 27 (35.5%) of the 76 subjects enrolled in the Group 1 achieved MDA. All the Group 2 subjects were continuatively in MDA during the same time period. **Figure 4** shows that, whereas no significant changes in ESR, CRP, haemostatic and fibrinolytic variables occurred in the Group 2, during the 6-months treatment with TNF-α blockers, both ESR and CRP significantly changed in Group 1. Haemostatic and fibrinolytic variables changed in a similar manner.

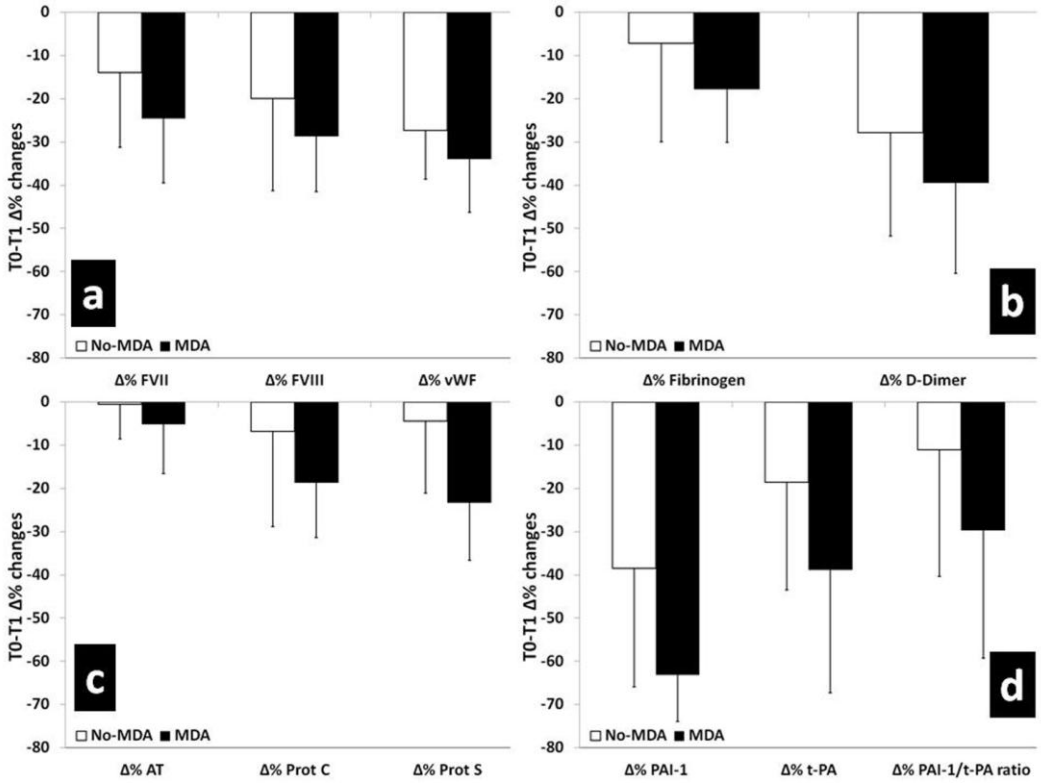
Figure 4. Changes in haemostatic and fibrinolytic parameters during the 6-month follow-up.



In particular, Δ%PAI (r=0.326, p=0.004), Δ%PAI-1/t-PA ratio (r=0.283, p=0.013), Δ%DD (r=0.250, p=0.029) showed a direct significant correlation with Δ%CRP in the Group 1 subjects, but not in those of the Group 2.

By stratifying the Group 1 population according to the achieving of MDA, Δ% of haemostatic and fibrinolytic variables levels were significantly higher in those achieving MDA than in those that did not (**Figure 5**).

Figure 5. % changes from baseline to T1 in patients receiving TNF-α blockers stratified according to Minimal disease activity (MDA) achievement.

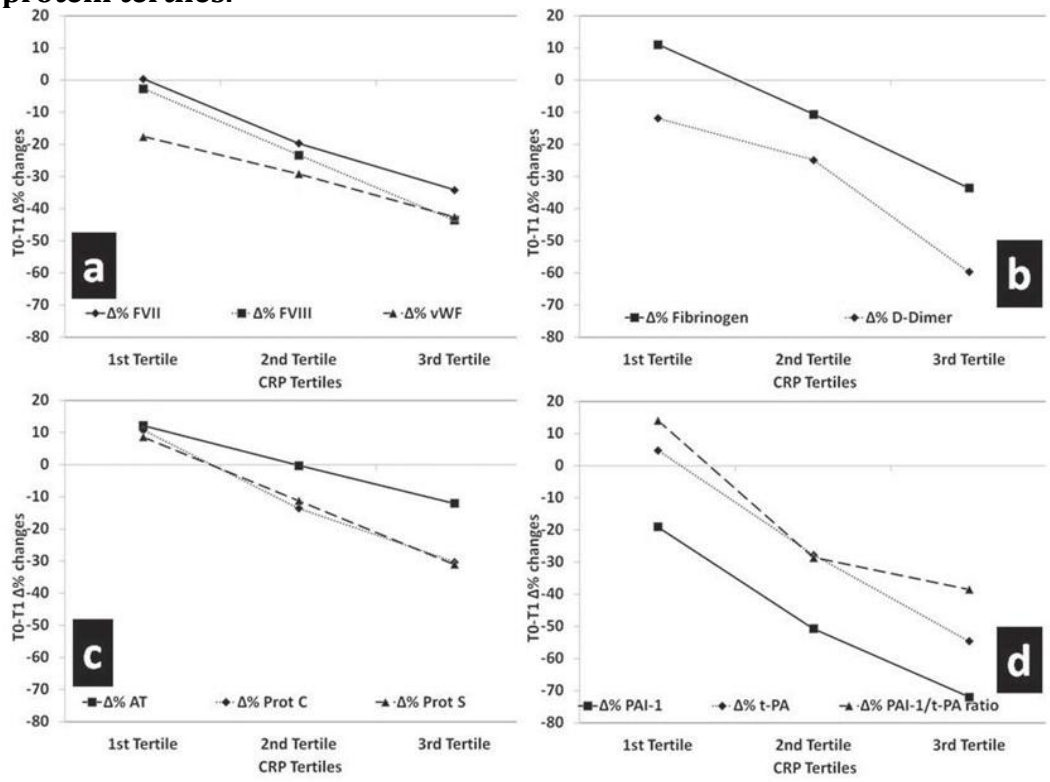


To address the relationship between inflammation and changes in haemostatic and fibrinolytic variables, when the Group 1 population was stratified according to Δ%CRP tertiles, progressively higher Δ% changes in haemostatic and fibrinolytic variables were found for increasing Δ%CRP tertiles (**Figure 6**). Further refining these results, in a linear regression model, after adjusting for all the other variables, Δ%CRP significantly

predicted changes in PAI-1 (β=0.691, p<0.001), in t-PA (β=0.326, p=0.004) and in D-Dimer (β=0.233, p = 0.008).

These same multivariate analysis, performed in Group 2 patients, did not retrieve any significant result.

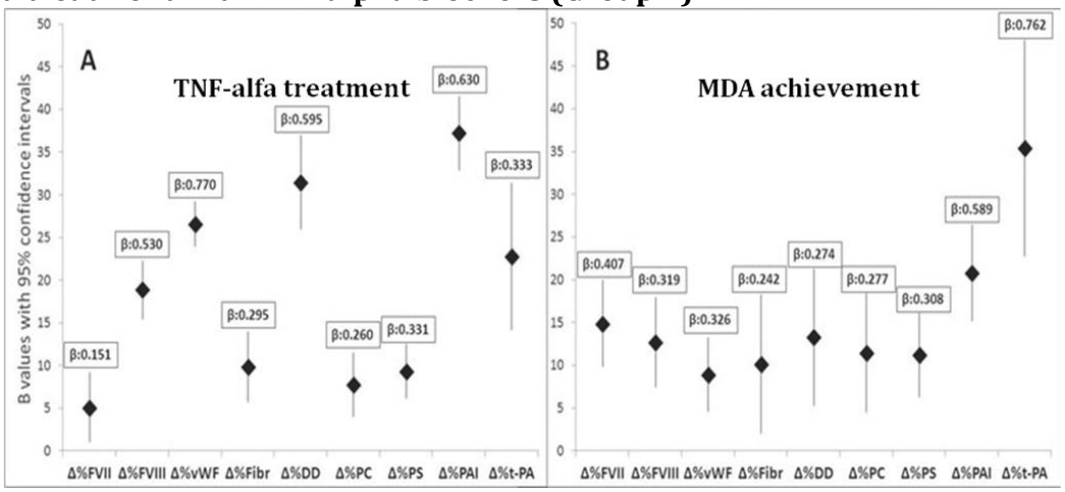
Figure 6. % changes in haemostatic and fibrinolytic variables in patients receiving TNF-α blockers stratified according to C-reactive protein tertiles.



In multiple linear regression analyses on the whole study sample (Group 1 and Group 2), the treatment with TNF-alpha blockers determined significantly higher changes in haemostatic and fibrinolytic variables as compared to those found in subjects continuatively treated with traditional DMARDs (**Figure 7, Panel A**).

Specifically analyzing subjects receiving a treatment with TNF-alpha blockers (Group 1), the MDA achievement was a predictor of higher changes in haemostatic and fibrinolytic variables as compared with no-MDA status (**Figure 7, Panel B**).

Figure 7. Multivariate analysis for the prediction of changes in haemostatic and fibrinolytic variables. Panel A: Effect of the treatment with TNF-alpha blockers versus traditional DMARDs in the whole study sample (Group 1 and Group 2). Panel B: Effect of Minimal Disease Activity (MDA) achievement versus no-MDA status in subjects receiving a treatment with TNF-alpha blockers (Group 1).



Overall, results of these multivariate analyses showed that a 6-month treatment with TNF-alpha blockers impacted on fibrinolysis parameters (PAI-1 and t-PA) as well as on some acute phase proteins (D-Dimer, FVIII and vWF). In contrast, the MDA achievement during treatment with TNF-alpha blockers maximally impacted on fibrinolytic parameters (PAI-1 and t-PA).

Of interest, same results were confirmed when analyzing standardized β values.

Results of this prospective study provide further evidence about the link between inflammation and thrombotic risk. In particular, we documented that the control of the inflammatory process is associated with a significant improvement of haemostatic and fibrinolytic parameters in PsA subjects, maximal changes being documented in patients achieving MDA. These variables have been found to predict arterial and venous thrombosis, that are major complications in PsA.[64] Previous studies have already shown that the overproduction of pro-inflammatory cytokines (TNF-α, IL-6), besides playing

a crucial role in the inflammatory process correlated with rheumatic disease activity,[65] it is also involved in the modulation of the fibrinolytic system.[66] The total fibrinolytic potential of human blood is determined by the balance between plasminogen activators (t-PA) and plasminogen activator inhibitors (PAI-1).[66] TNF-α has proved to be a potent agonist of PAI-1 expression and regulation.[67] In addition, high plasma levels of prothrombin fragment 1+2 (F1 + 2) and of D-dimer (markers of thrombin activation and of fibrinolysis, respectively) have also been found in RA patients.[68] Thus, by inducing a pro-coagulant shift in the haemostatic balance, chronic inflammation promotes fibrin generation and, in turn, thrombosis.[69,70]

At variance with all the other previous studies, exclusively focused on RA patients, we have extended the evaluation of global haemostasis changes to PsA patients.

In addition, besides the evaluation of fibrinolytic balance, in this study we reported about changes in a series of other hemostatic factors. In detail, vWF, FVIII and FVII are recognized acute phase proteins, increasing in the presence of inflammation, cancer and pregnancy[71] and leading to a prothrombotic state and to an increased risk of venous and arterial thrombosis.[72] Moreover, Prot. C and Prot. S are natural anticoagulant proteins that play a major role in opposing hypercoagulable states.[73] Consistent with the link between natural anticoagulants and variables involved in hypercoagulable states, changes we have reported in Prot. C and Prot. S levels are likely to be related to the changes that occurred in PAI-1 and t-PA levels.

This is also the first study prospectively comparing the effects induced by TNF-α inhibitors and by traditional DMARDs on secondary haemostasis parameters. Indeed, the contemporary evaluation of a group of subjects continuatively treated with traditional DMARDs allowed for a comparison of the effects of traditional and biologic anti-rheumatic drugs on hemostatic parameters.

In line with previous studies,[74] at the baseline evaluation, by assessing the impact of inflammation and of disease activity on haemostatic and fibrinolytic variables, we found that most of these parameters were significantly higher in patients with an active disease than in those with minimal disease activity. Of interest, being all subjects under DMARDs at the time of the baseline assessment, we are confident to have evaluated the impact of disease activity on haemostatic and fibrinolytic balance.

In addition to traditional DMARDs, treatment with TNF-α inhibitors improved clinical and laboratory measures of disease activity in rheumatic diseases and reduces local and systemic inflammation. Besides the control of inflammation, TNF-α inhibitors have been found to down-regulate fibrinolytic as well as haemostatic parameters and to normalize platelet hyper-reactivity, thus leading to a reduction in the CV risk.[61,66,75,76]

We have found that, at the end of the 6-month follow-up, as compared with those continuatively treated with DMARDs, those starting a treatment with TNF-α blockers, showed a significant reduction in haemostatic and fibrinolytic parameters. In addition, maximal changes in coagulation variables were found in those achieving the MDA during the TNF-α blocker

treatment. In keeping with this, for increasing changes in CRP levels, a progressively higher variation in all evaluated variables was found.

For comparison purposes, inflammatory markers and hemostatic/fibrinolytic parameters did not change in the group of patients kept on receiving traditional DMARDs (all changes <10% from baseline).

In addition, with the exception of AT levels, DMARDs patients had higher levels of haemostatic and fibrinolytic factors when compared to anti-TNF patients (both to those achieving MDA and to those with persistent active disease). These findings might suggest the possibility that the mechanism of action of TNF-α inhibitors on haemostatic and fibrinolytic parameters does not depend only from the successful control of inflammation (MDA achievement). However, this issue cannot be ruled out in this study and further data are needed to understand whether improvements obtained in hemostatic and fibrinolytic parameters are just correlated to reduction of inflammatory status.

In conclusion, our study confirms the role of TNF-α inhibition in the reduction of systemic inflammation, with a significant improvement in the global hemostatic and fibrinolytic balance of PsA patients. These data suggest a potential cardio-protective effects of TNF-α inhibitors. Whether this is a drug-specific effect or a consequence of the inflammatory process control is unclear so far and cannot be ruled out based on the present data.

Further studies evaluating risks (side effects)[77] and benefits (anti-inflammatory and cardioprotective effects) of the treatment with TNF-α inhibitors are needed to allow for a tailored anti-rheumatic treatment choice.

SURROGATE MARKERS OF ATHEROSCLEROSIS

Sub-clinical atherosclerosis: Carotid intima-media thickness

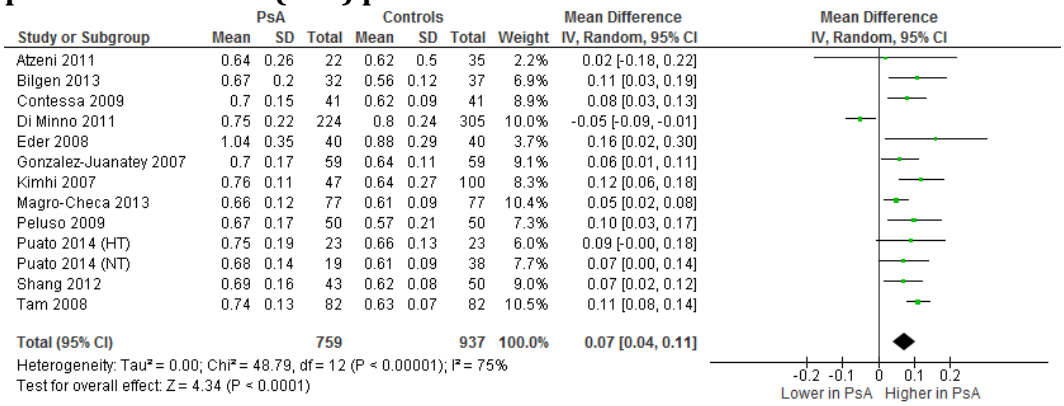
Carotid intima-media thickness (IMT) assessment is a non-invasive imaging test for subclinical atherosclerosis,[22,23] and it has been widely accepted as one of the strongest predictors of major CV events (stroke, myocardial infarction, heart failure, or CV death).[24,25] The presence of carotid plaques is considered an even more reliable predictor of CV events than IMT.[26] Thus, these surrogate markers of subclinical atherosclerosis provide important prognostic information over and above traditional CV risk factors. Some functional and ultrasonographic assessments support the evidence of an increased CV risk profile in PsA patients. In a cohort study, PsA patients showed an higher carotid IMT than controls (0.76±0.11 vs 0.64±0.27, p<0.001).[78] To avoid potential confounders, Gonzalez-Juanatey et al studied a population of PsA subjects without established VRFs. Compared with matched controls, an impaired endothelium-dependent vasodilation (p=0.008) and a higher IMT (p=0.031) were found in the PsA group.[79,80]

Consistent with data showing a correlation between inflammation and IMT,[60,81] an association between disease activity in PsA and the presence of carotid plaques has been reported.[82]

With the aim to provide a pooled analysis on the topic of sub-clinical atherosclerosis in PsA patients, we performed a systematic review and meta-analysis of literature studies.[83] In 12 studies (13 data-sets on 759 cases and 937 controls), we found a significantly higher CCA-IMT in 759 PsA patients than in 937 controls (MD: 0.07 mm; 95%CI: 0.04, 0.11; P <0.0001, **Figure 8**). The heterogeneity among studies was significant (I^2=75%; P<0.00001) but,

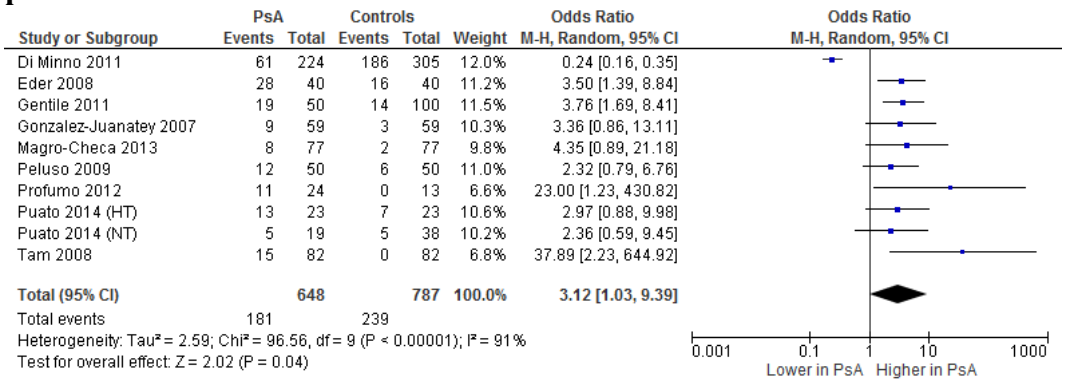
after excluding one study, similar results were obtained without heterogeneity (MD: 0.08 mm; 95%CI: 0.07, 0.10; P <0.00001, I^2=2%; P<0.00001).

Figure 8. Common carotid artery intima-media thickness (CCA-IMT) in psoriatic arthritis (PsA) patients and controls.



A total of 9 studies (10 data-sets on 648 cases and 787 controls), showed an increased prevalence of carotid plaques in 648 PsA patients as compared to 787 controls (28.3% vs 10.9%), with a corresponding OR of 3.12 (95%CI: 1.03, 9.39; P=0.04, **Figure 9**). Significant heterogeneity among studies was found (I^2=91%; P<0.00001). Of interest, after excluding one study, we found similar results without heterogeneity (OR: 3.51; 95%CI: 2.33, 5.29; P <0.00001, I^2=0%; P=0.67).

Figure 9. Prevalence of carotid plaques in psoriatic arthritis (PsA) patients and controls.



With the aim to evaluate effects of different treatments on IMT, we performed a case-control study[76] on 224 PsA patients (120 on TNF-α blockers and 104 on traditional Disease Modifying Anti-Rheumatic Drugs [DMARDs]) that underwent a CCA-IMT ultrasound assessment. As many as 305 matched subjects without any inflammatory/rheumatologic disease, served as controls. As shown in **Table 4**, PsA subjects without VRFs had a CCA-IMT ($_{mean\text{-}max}$CCA and $_{mean\text{-}max}$Bulb) significantly higher than that of control subjects. Among those with 1-3 risk factors, the CCA-IMT of PsA subjects was significantly lower than that of controls, while in subjects with ≥3 VRFs, no significant difference was present between PsA and control subjects.

Table 4. Intima-media thickness (CCA and Bulb) in subjects with psoriatic arthritis (PsA) according to the number of vascular risk factors (VRFs).

Number of VRFs	$_{mean\text{-}max}$CCA (mm)			$_{mean\text{-}max}$Bulb (mm)		
	PsA N= 224	Controls N= 305	p value	PsA N= 224	Controls N= 305	p value
0 VRF n=	0.72±0.25 20	0.56±0.08 24	0.025	1.09±0.40 20	0.87±0.21 24	0.032
1 VRF n=	0.69±0.24 60	0.78±0.14 67	0.001	0.96±0.44 60	1.24±0.34 67	<0.0001
2 VRF n=	0.70±0.17 52	0.78±0.19 90	0.006	1.02±0.50 52	1.29±0.41 90	<0.0001
3 VRF n=	0.75±0.15 44	0.86±0.31 74	0.040	1.09±0.35 44	1.37±0.45 74	<0.0001
4 VRF n=	0.88±0.25 44	0.88±0.31 39	0.833	1.25±0.43 44	1.39±0.43 39	0.126
5 VRF n=	0.91±0.13 4	0.96±0.19 11	0.720	1.37±0.65 4	1.42±0.37 11	0.633

Note: all p are for log-transformed values of CCA and Bulb; VRFs: vascular risk factors; N= number of subjects; CCA: common carotid artery

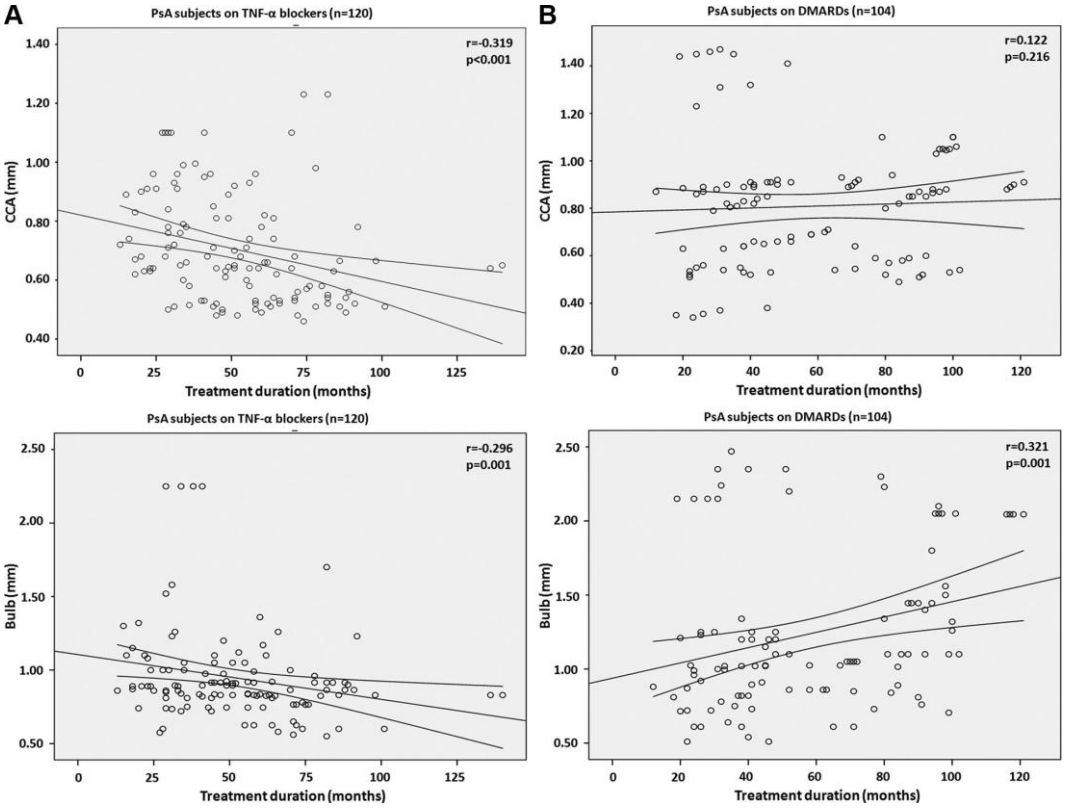
As showed in **Table 5**, PsA subjects on TNF-α blockers and those on traditional DMARDs significantly differed as to CCA-IMT measurements. Accordingly, carotid plaques were found in 15.8% of subjects on TNF-α blockers and in 40.4% of subjects on traditional DMARDs (p<0.0001).

Table 5. Demographic and clinical features of the study population (Panel A) and stratification according to treatment schedules (Panel B).

Variable	PsA subjects on TNF-α blockers n=120	PsA subjects on DMARDs n=104	p
Age	51.5±10.9	53.8±11.9	0.120
Male sex	56 (46.7%)	48 (46.2%)	1.000
MetS	36(30.0%)	40 (38.5%)	0.204
IFG	12(10.0%)	8(7.7%)	0.642
Hypertension	48(40.0%)	44(42.3%)	0.786
Hypercholesterolemia	68(56.7%)	48(46.2%)	0.140
Hypertriglyceridemia	28(23.3%)	32(30.8%)	0.229
Obesity	76(63.3%)	64(61.5%)	0.784
Smoking habit	36(30%)	28(26.9%)	0.658
Disease duration (months)	113.62±58.24	103.79±85.91	0.312
Treatment characteristics			
Treatment duration (months)	52.33±24.11	58.22±29.21	0.100
Disease activity			
Low disease activity	88 (73.3)	30(28.8)	0.001
ESR (mm)	14.23±8.53	24.11±16.66	<0.0001
CRP mg/dl	1.98±1.8	3.6±3.9	0.007
IL-6 (ng/L)	3.9±0.6	5.0 ± 0.8	0.07
Ultrasound evaluation			
$_{mean\text{-}max}$CCA (mm)	0.70±0.18	0.80±0.26	0.002
$_{mean\text{-}max}$Bulb (mm)	0.94±0.31	1.24±0.52	<0.001
Carotid plaques n(%)	19(15.8%)	42(40.4%)	<0.0001

The $_{mean\text{-}max}$Bulb showed a direct correlation with treatment (r=0.321, p=0.001) and with disease (r=0.392, p<0.001) duration in those on traditional DMARDs (**Figure 10)**. In the same group, $_{mean\text{-}max}$CCA showed a direct correlation with disease duration (r=0.203, p=0.039). In keeping with this, subjects with ultrasound evidence of carotid plaques had a disease duration significantly longer than that of subjects without (130.34±106.75 vs 101.09±52.56, p=0.007). No correlation with treatment duration was documented in PsA subjects on DMARDs when the $_{mean\text{-}max}$CCA was examined. In those on TNF-α blockers, whereas no significant correlation was found between disease duration and C-IMT ($_{mean\text{-}max}$CCA: r=-0.005, p=0.954; $_{mean\text{-}max}$Bulb: r=0.011, p=0.904), a significant inverse correlation between treatment duration and C-IMT was found ($_{mean\text{-}max}$CCA: r=-0.319, p<0.001; $_{mean\text{-}max}$Bulb: r=-0.296, p=0.001). The presence of carotid plaques inversely correlated with treatment duration (r=-0.206, p=0.024), and did not show a significant correlation (r=0.014,p=0.877) with disease duration.

Figure 10. Scatter plot of Pearson's correlations between C-IMT (mm) and treatment duration (months). A, TNF-α blocker group. B, Traditional DMARDs group. All *P* values are for log-transformed values of CCA and bulb.



In a linear regression model, age (β=0.382, p<0.0001), male sex (β=0.134, p=0.009), treatment with traditional DMARDs (β=0.202, p=0.001) and clinical remission (β=-0.236, p<0.0001) independently predicted the CCA-IMT (whole PsA sample). Among VRFs, hypertension (β=0.221, p<0.0001) independently predicted the C-IMT in this setting as did the number of VRFs (β=0.225, p<0.0001).

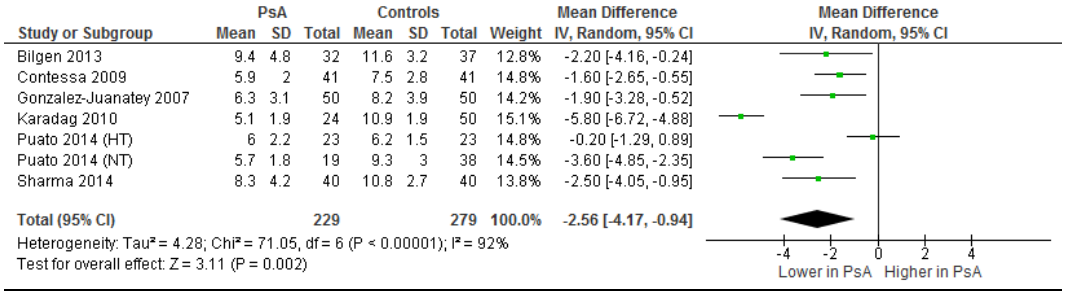
When the patient population was stratified according to treatment schedules, whereas age predicted CCA-IMT both in the DMARDs group (β=0.371, p<0.0001) and in the TNF-α-blockers group (β=0.302, p<0.0001), ESR levels directly predicted CCA-IMT (β=0.254, p=0.001) in PsA subjects on DMARDs, but not in those on TNF-α blockers. In keeping with this, arterial hypertension predicted the C-IMT only in subjects on DMARDs treatment (β=0.646, p<0.0001). On the other hand, treatment duration significantly and independently (β=-0.317, p<0.0001) predicted the CCA-IMT in those on TNF-α blockers, but not in those on traditional DMARDs.

The vascular reactivity: Flow-mediated and Nitrate-mediated dilation

Flow-mediated dilation (FMD) and nitrate-mediated dilation (NMD) are considered surrogate markers of subclinical atherosclerosis and independent predictors of CV events,[84] thus providing important prognostic data over and above traditional CV risk factors.

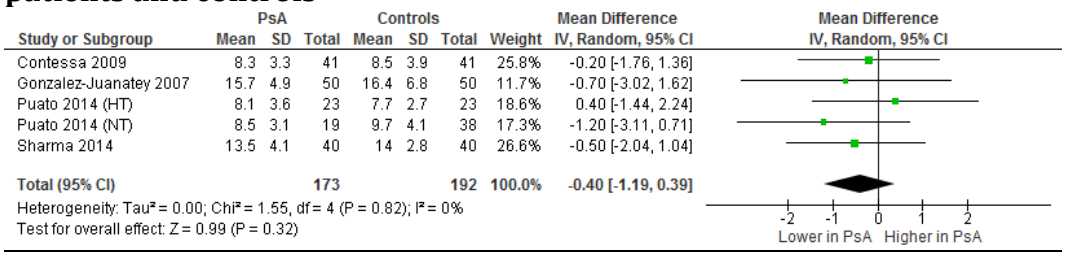
In the frame of our project, we have performed a meta-analysis[83] in which we pooled together 6 studies (7 data-sets on 229 PsA patients and 279 controls), showing a significantly lower FMD in PsA subjects as compared to controls (MD: -2.56%; 95%CI: -4.17, -0.94; P=0.002, **Figure 11**).

Flow 11 mediated-dilation (FMD) in psoriatic arthritis (PsA) patients and controls.



In contrast, no difference in the NMD was found in 4 studies (5 data-sets on 173 cases and 192 controls) with a MD of -0.40% (95%CI: -1.19, 0.39, P=0.32, **Figure 12**).

Figure 12. Nitrate mediated-dilation (NMD) in psoriatic arthritis (PsA) patients and controls



Our results consistently show that PsA is associated with subclinical atherosclerosis and endothelial dysfunction. In particular, we demonstrated an increased carotid CCA-IMT with a high prevalence of carotid plaques and impaired FMD in patients with PsA. Our findings are strengthened by meta-regression models providing the evidence that demographic variables (age and gender) did not influence the impact of PsA on carotid CCA-IMT.

Overall, these data clearly show an increased CV risk in patients with PsA and suggest the need for a strict monitoring of CV risk factors and of signs of subclinical atherosclerosis in PsA patients. Accordingly, previous published studies reported an increased risk of major CV events and CV death in patients with PsA.

Many CV risk factors are thought to have a causal role in the atherosclerotic process.[85] Although PsA patients exhibit an increased prevalence of these CV risk factors, the relationship between subclinical atherosclerosis and PsA seems to be more complex and the presence of traditional risk factors might not entirely explain the accelerated atherosclerotic process in this clinical setting.[86] Thus, other mechanisms (i.e. inflammatory and immunological) have been proposed to explain the relationship between PsA and atherosclerosis and immune-mediated inflammation seems to play a pivotal role in the pathogenesis of atherosclerosis, being involved in endothelial dysfunction, plaque rupture and thrombosis.[87] In keeping with this, some common markers of inflammation (i.e. CRP, fibrinogen) are emerging predictors of CV disease,[88,89] and patients with PsA exhibit elevated levels of these acute-phase proteins.[90] ESR, another marker of inflammation, is

commonly found increased in PsA and high ESR has been associated with increased overall mortality in this clinical setting.[91]

The link between inflammation and the subclinical atherosclerosis has been further addressed in our cross-sectional study showing that, in the absence of VRFs, the CCA-IMT of PsA patients is significantly higher than that of controls. However, when stratified according to the presence of VRFs, the CCA-IMT of PsA individuals is lower than those of controls. The finding that, in the presence of 1-3 VRFs, PsA subjects have a lower C-IMT as compared with controls, may be taken to suggest a protective effect of anti-inflammatory drugs on the CCA-IMT. On the other hand, since an higher CCA-IMT is predicted by an increasing number of VRFs in PsA subjects, an additive effect of VRFs can be postulated to overcome the protective effect of anti-inflammatory drugs. However, the CCA-IMT was lower in PsA subjects on TNF-α blockers than in those on traditional DMARDs and the co-existence of a MetS was associated with an higher CCA-IMT in subjects on DMARDs than in those under TNF-α blockers. Over the past decade, TNF-α blockers have provided a significant advancement for preventing the progression of inflammation and of the structural damage of joints in patients with psoriasis, PsA, or RA.[92,93,94] In addition, TNF-α blockers improve the endothelial function and the vascular risk profile in patients with immune-mediated disorders.[95] In our study, the effects elicited by TNF-α blockers appear to be accounted for by subtle anti-inflammatory mechanisms. ESR levels significantly predicted CCA-IMT only in PsA subjects on DMARDs. ESR and CRP were significantly lower in subjects on TNF-α blockers than in those on DMARDs. By inhibiting pro-inflammatory cytokines involved in insulin

regulation, lipid metabolism and in body weight homeostasis, TNF-α blockers reduce the prevalence of the MetS in subjects with rheumatologic disease.[96] Consistent with a large body of evidence, as much as 35-40% of the present PsA sample exhibited the MetS. However, at variance with subjects on DMARDs, the MetS was not associated with differences in CCA-IMT in subjects on TNF-α blockers. Accordingly, clinical remission was present in 73.3% of subjects on TNF-α blockers and in 28.8% of those on traditional DMARDs. Consistent with the concept that the reduction of systemic inflammation may hamper the cascade that leads to enhanced vascular risk in PsA patients, our results documented a significantly reduced CCA-IMT in patients on TNF-α blockers as compared to those on DMARDs. In addition, our data show that, while largely independent of disease duration and preexisting atherogenic risk factors featuring the MetS, the efficacy of TNF-α blockers was related to treatment duration. This is consistent with a progressive effect of inflammation on the CCA-IMT.

Overall, our findings are in line with some experimental and clinical evidence, supporting the hypothesis that premature atherosclerosis may be one of the main features of PsA and that chronic inflammation plays an important role in its pathogenesis, acting independently and/or synergistically with traditional CV risk factors. The clinical relevance of our results can be better understood when we consider that the risk of myocardial infarction increases of 43% every 0.163 mm increase in carotid IMT[97] and that the prevalence of carotid plaques is an even more reliable predictor of CV events than IMT.[26] Moreover, each 1% decrease in FMD has been associated with a 12% increase of cardiovascular events.[98]

Our results further support the need for large long-term interventional trials with CV end-points to investigate whether benefits in articular disease achieved by aggressive suppression of inflammation may translate into reduced CV risk in PsA.

In the meanwhile, patients with PsA may benefit from a periodic assessment of surrogate markers of CV risk to establish more specific CV prevention strategies for these patients.

Conclusions and Perspectives

Overall, results of our project suggest that PsA patients have a increased platelet reactivity, impaired fibrinolysis and subclinical atherosclerosis accompanied by an impaired endothelial function.

Most of these alterations seem to be associated with the inflammatory status and are likely to be modified by the anti-rheumatic treatments.

All these data are in line with many literature data that support the possibility of an increased CV risk in patients with rheumatic diseases. The finding that the vascular morbidity/mortality of rheumatic patients resembles that of type II diabetes, further helps to define the severity of the CV risk in this clinical setting.[14] However, the difference documented in the risk of CV death between community-based and clinic-based studies, argues for different risk according to the disease activity, only those with a severe disease being referred to specialized centers.[39, 40] The extent to what the estimate of the CV risk in rheumatic patients is likely to be biased by a significant under-diagnosis, and in turn by under-treatment, of concomitant VRFs is unknown so far and needs to be evaluated.[99]

The increase of CV risk in patients with rheumatic diseases as compared with both healthy populations and VRFs-matched subjects support the notion that systemic inflammation acts as an independent CV risk factor.[100] The improvement of the CV risk profile following the control of systemic inflammation by anti-inflammatory treatments argues for this possibility as well.[101,102] While this implies that the incidence of CV morbidity/mortality should be re-evaluated according to an optimal inflammation control (i.e. achieving minimal disease activity), inflammation/disease activity has been

recently suggested to be included in the CV risk factor profile of such patients.[100] However, scores currently used for the general population (e.g. the Framingham score; the Systematic Coronary Risk Evaluation model),[45, 46] do not take into account the role of inflammation.[47] Based on standard algorithms, the European League Against Rheumatism (EULAR)[1] suggested the application of a 1.5 multiplier to the risk calculated in rheumatic patients. While appealing for its simplicity, this approach requires a long-term validation in which repeated CV risk assessments in rheumatologic settings are mandatory.[1]

In the meanwhile, searching to explain mechanism underlying the CV risk in PsA patients, we have designed a further project which has been funded by the Italian Ministry Health. In this prospective study we will assess, at different time-points, changes in the circulating transcriptome and in carotid CCA-IMT during treatment with TNF-alpha blockers. The main objective is to assess if treatment with TNF-alpha blockers may reduce CCA-IMT in patients with active PsA. Another major goal is to identify circulating transcriptomic markers associated with treatment response. Results of this study will provide information about the link between inflammation and subclinical atherosclerosis, allowing for a better definition of the CV risk profile in PsA patients. In addition, we will try to identify circulating markers predicting the minimal disease activity (MDA) achievement.

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