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Coordinatore: Prof. Giovanni Di Minno

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CHANGES IN ENDOTHELIAL FUNCTION IN PATIENTS WITH PSORIATIC ARTHRITIS TREATED WITH APREMILAST. A 1-YEAR PROSPECTIVE COHORT STUDY.

TUTORE
Ch.mo Prof.
Giovanni Di Minno

CANDIDATO
Dott. Pasquale Ambrosino

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1. BACKGROUND

With a prevalence of 0.3-1% in the general population, psoriatic arthritis (PsA) is one of the most common chronic inflammatory diseases worldwide [1]. This condition affects up to 40% of patients with psoriasis, leading to a chronic arthropathy with disability and severe physical limitations [2].

There is growing acknowledgement that the inflammatory state associated with PsA is not limited to the joints but also impacts other organs and systems [3]. Recent findings about the inflammatory mechanisms that determine insurgence and progression of PsA have been accompanied by the increasing awareness that patients with this condition often have comorbidities associated with chronic inflammation, including osteoporosis, gout, inflammatory bowel disease and, most important, cardiovascular (CV) disease [4,5].

1.1 Cardiovascular comorbidity in PsA patients

Several studies documented an increased CV risk in PsA patients, with increased prevalence of myocardial infarction, stroke and CV death [6,7]. Based on meta-analytical data, patients with psoriasis and PsA have 29% and 55% higher risk of incident myocardial infarction, respectively [8,9]. Accordingly, a recent meta-analysis documented a 27% higher risk of any stroke (both ischemic and hemorrhagic) among PsA patients [10]. Compared with the general population, 62% increased mortality has also been reported in PsA subjects, with CV disease accounting for approximately 36% of the excess mortality in this clinical setting [5,11].

The nature of the relationship between PsA and CV risk remains to be elucidated [12]. To date, robust evidence suggests that immune-mediated inflammation and oxidative stress are key elements in the development and progression of the atherosclerotic process [13]. A number of pro-inflammatory cytokines are involved in systemic and local inflammation. Among them, tumor necrosis factor-α (TNFα) upregulates adhesion molecules, leading to the

formation of fatty streaks in the vessel wall and thus contributing to the onset of atherosclerosis [14]. Interleukin-6 (IL-6) is another pro-inflammatory cytokine called into question to explain the increased CV risk of PsA patients. IL-6 stimulates hepatic synthesis of C-reactive protein (CRP) and fibrinogen [15], being these acute-phase proteins emerging predictors of CV disease [16,17]. Moreover, IL-6 is able to amplify endothelial cell adhesiveness by activating the production of tissue factor, fibrinogen and factor VIII [18]. Erythrocyte sedimentation rate (ESR), another marker of inflammation, is commonly found increased in PsA [19], with high ESR levels being associated with increased overall mortality among patients with rheumatic diseases [20]. The strong correlation between these markers of inflammation and those of platelet activation (CD62P, CD63) suggests that disease activity may be responsible for platelet hyper-reactivity in PsA [21]. In line with these data, a direct correlation between inflammatory status (as expressed by CRP levels) and increasing quartiles of maximal platelet aggregation has also been documented in subjects with PsA [22]. Another element that may represent the link between atherosclerosis and PsA is asymmetric dimethylarginine (ADMA), a competitive inhibitor of nitric oxide (NO) synthesis [23]. Elevated levels of ADMA have been identified as predictors of CV disease [24], and plasma levels of this molecule have been found significantly higher in PsA patients as compared to healthy subjects [25].

While the increased CV risk in psoriatic patients is generally attributed to the chronic systemic inflammation related to skin and joint disease, it should also be considered that an increased prevalence of the traditional CV risk factors (VRFs) may significantly contribute to the atherosclerotic burden in this clinical setting [13]. In fact, PsA patients show a higher prevalence of metabolic syndrome (MetS) compared to the general population and even to other rheumatic diseases, such as rheumatoid arthritis or ankylosing spondylitis [26]. Accordingly, an increased prevalence of hypertension, hyperlipidaemia, obesity, and type II

diabetes has been found in PsA patients as compared with those who have only psoriasis [4]. Liver steatosis, a recognized CV risk marker [27], is also frequent in PsA patients [28]. In addition to traditional VRFs, increased homocysteine levels have also been documented in PsA, maybe as a consequence of some medications (methotrexate and sulfasalazine) commonly used in the treatment of PsA [29].

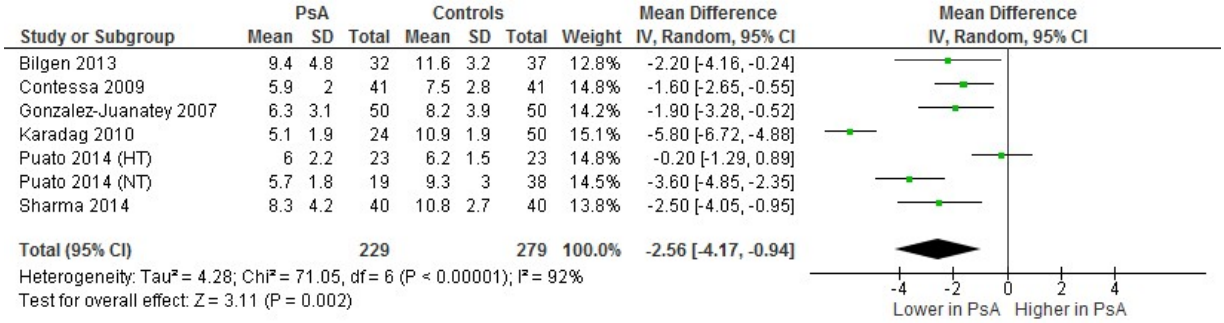
Overall, a large number of experimental and clinical evidence supports the hypothesis that premature atherosclerosis may be one of the main features of PsA and that chronic inflammation plays an important role in its pathogenesis, acting independently and/or synergistically with traditional VRFs

1.2 Cardiovascular risk markers in PsA patients

To further address the issue of the increased CV risk among PsA patients, a growing attention has been given to the assessment of the association between PsA and subclinical atherosclerosis, a recognized marker of CV disease [30].

Endothelial dysfunction is the earliest stage of the atherosclerotic process and even a trigger of CV events [31]. Flow-mediated dilation (FMD) is widely accepted as an accurate and non-invasive method to assess endothelial function in humans [32] and, in turn, as a surrogate marker of subclinical atherosclerosis. Moreover, FMD is currently considered an independent predictor of CV events [33], thus providing important prognostic data over and above traditional CV risk factors. A meta-analysis has recently demonstrated that patients with PsA display impaired endothelial function, as expressed by lower FMD values, when compared to age-matched controls (**Figure 1**) [12]. Accordingly, a series of case-control studies conducted in PsA patients by using other surrogate markers of CV risk reported accelerated carotid atherosclerosis [25,34] and increased arterial stiffness [35,36] in patients with PsA.

Figure 1. Flow mediated-dilation (FMD) in psoriatic arthritis (PsA) patients and healthy controls.



***Modified from**: Di Minno MND, Ambrosino P, Lupoli R, Di Minno A, Tasso M, Peluso R, Tremoli E. Cardiovascular risk markers in patients with psoriatic arthritis: a meta-analysis of literature studies. Ann Med. 2015;47:346-353.*

1.3 Anti-inflammatory therapy and cardiovascular risk

The awareness of an increased atherosclerotic burden among PsA patients brought into question the impact that inflammatory suppression may have on CV risk in this setting. Since inflammation plays a key role in the pathogenesis of all rheumatic diseases, including PsA, and considering its pivotal role in driving all stages of atherosclerosis, it is mandatory to hypothesize that controlling inflammation may provide beneficial effects on CV risk in these patients [37].

If the use of cicloxygenase-2 (COX2) inhibitors and traditional non-steroidal anti-inflammatory drugs (NSAIDs) is associated with an increased CV risk in the general population [38], methotrexate has been associated with reduction of foam cell formation, down-regulation of adhesion molecule expression on endothelial surface, reduced synthesis of atherogenic cytokines (TNFα, IL-6, and IL-1), and reduced risk of MetS [37]. Moreover, methotrexate has shown to reduce the incidence of myocardial infarction in responders to therapy [39].

Similarly, based on meta-analytical data, the use of TNFα-blockers in rheumatic patients has been associated with a 30% relative reduction in all CV events and a 41% reduction of myocardial infarction in comparison to traditional disease-modifying antirheumatic drugs

(DMARDs) [40]. In a retrospective study, the use of abatacept was associated with an even lower risk of myocardial infarction when compared to TNFα-blockers [41]. In contrast, in 2011, both European and U.S. agencies withdrew approval of the anti-IL-12/23 human monoclonal antibody briakinumab (used for the treatment of moderate to severe chronic plaque psoriasis) due to the high incidence of major CV events [42].

Several studies focused on the impact of DMARDs and biologic agents on CV risk markers, such as FMD. A recent meta-analysis showed that TNFα-blockers are able to improve FMD in patients with rheumatoid arthritis, thus contrasting endothelial dysfunction in this clinical setting [43]. Similar results on endothelial function were reported in a small sample of patients with PsA [44]. Accordingly, other studies showed that TNFα-blockers may improve aortic stiffness in patients with inflammatory arthritis [45], and even reverse carotid atherosclerosis progression in patients responding to treatment [46]. Similarly, inhibition of IL-6 has been associated with improvement of FMD and arterial stiffness in other studies on the use of tocilizumab in rheumatoid arthritis [47]. In contrast, despite animal studies showed a favourable effect of abatacept on atherosclerosis [48], this drug in humans was not associated with an improvement of surrogate measures of subclinical atherosclerosis [49].

Overall, most evidence (sometimes contrasting) on the impact of anti-inflammatory therapies on the risk of CV events and on markers of subclinical atherosclerosis come from patients with rheumatoid arthritis or psoriasis, while only a few data are currently available on CV consequences of controlling inflammation in patients with PsA. Thus, while a great amount of data are currently available on traditional DMARDs or TNFα-blockers, little is known about the CV effects of the drugs commonly used for the treatment of PsA, such as anti-IL-17, anti-IL-12/23 or the new oral targeted synthetic DMARD, namely Apremilast (Otezla®).

2. AIMS

In 2014, Apremilast (Otezla®), a novel orally administered phosphodiesterase 4 (PDE4) inhibitor, was approved for treatment of active PsA and moderate to severe plaque psoriasis [50]. By inhibiting PDE4, apremilast elevates intracellular levels of cyclic adenosine monophosphate (cAMP), thus regulating the expression of several inflammatory mediators [51]. In patients with PsA, treatment with apremilast (30 mg twice daily) has shown to decrease plasma levels of pro-inflammatory cytokines, including TNFα, IL-6, IL-8, macrophage inflammatory protein-1β (MIP-1β), monocyte chemoattractant protein-1 (MCP-1), ferritin, IL-17 and IL-23, at the same time increasing plasma levels of the anti-inflammatory cytokines IL-10 and IL-1R antagonist [51].

Besides its anti-inflammatory effect, PDE4 inhibition has shown to increase NO production by endothelial cells, hence improving endothelial function in animal models [52]. However, the impact of PDE4 inhibitors on clinical markers of vascular reactivity and endothelial function has not been evaluated in humans.

The aim of our study was to prospectively assess changes in FMD in a cohort of PsA subjects during a 12-month treatment with apremilast.

3. METHODS

From February 2017 to October 2019, consecutive patients diagnosed with PsA according to 2006 Classification Criteria for Psoriatic Arthritis [2] referring to the Rheumatology Unit of the Department of Clinical Medicine and Surgery, Federico II University Hospital, Naples, Italy were screened for enrollment in the present study. The major inclusion criteria were a diagnosis of PsA and the eligibility of patients to start a treatment with apremilast, namely an active disease with inadequate response or intolerance to a prior therapy with traditional disease-modifying antirheumatic drugs (DMARDs). Exclusion criteria were: age <18 years; acute myocardial infarction or stroke within the last 6 months; malignant disease (except basal cell carcinoma of the skin) under current chemotherapy regimens; any major surgical procedure during the 6 months prior to the first visit; inability to understand or sign the informed consent; suspicion of alcohol or drug abuse or any other condition associated with poor compliance in the investigator's opinion. Following the 5-day titration, patients fulfilling inclusion/exclusion criteria received the recommended maintenance apremilast dose of 30 mg taken orally twice daily. The study was conducted in accordance with the Declaration of Helsinki and submitted to our Institutional Review Board. All patients gave written informed consent.

Study protocol

After informed consent signature, a detailed medical history was recorded for each patient. Data about age, gender, previous and/or current medical conditions, current and past antirheumatic therapy, vascular risk factors (VRFs) were collected.

According to the National Cholesterol Education Program (NCEP) criteria,[53] abdominal obesity was defined as a waist circumference ≥102 cm for men and ≥88 cm for women; hypertriglyceridemia, as triglycerides levels ≥150 mg/dL; hypercholesterolemia as a total

cholesterol ≥200 mg/dL with or without high-density lipoprotein (HDL) cholesterol <40 mg/dL for men and <50 mg/dL for women; hypertension as a systolic blood pressure (SBP) ≥130 mmHg and/or diastolic blood pressure (DBP) ≥85 mmHg; impaired fasting glucose (IFG) as a fasting glucose ≥100 mg/dL.

All study procedures were performed in a temperature-controlled room (23°C), evaluating patients at study entry (T_0), after 3 months (T_{3m}), 6 months (T_{6m}) and 12 months (T_{12m}) of treatment with apremilast.

Study procedures

Main anthropometric, clinical and laboratory parameters were evaluated at different time-points.

1. Anthropometric parameters

Height was measured to the nearest 0.1 cm. Body weight was assessed by using an electronic beam scale with digital readout to the nearest 0.1 kg after emptying the bladder and with the subjects standing barefoot and wearing light indoor clothing. Body mass index (BMI) was calculated as body weight/(height^2).

2. Rheumatologic parameters

The Disease Activity Index for Psoriatic Arthritis (DAPSA) was assessed as a composite measure of disease activity in all included patients [54]. With a higher score representing a higher disease activity, DAPSA is based on the summation of the following five variables: 1. 68-Tender Joint Count (TJC68), which is the number of painful joints out of 68 when a standard amount of pressure is applied; 2. 66-Swollen Joint Count (SJC66), which is the number of swollen joints out of 66; 3. Patient Global Assessment (PtGA), and 4. Patient Pain

(PP) assessment, both based on a 10-cm visual analogue scale (VAS) representing the patient's self assessment of disease activity and pain, respectively, during the last week (scores ranging from 0-no activity/no pain to 10-very active/severe pain); and 5. C-reactive protein (CRP), expressed as mg/dl. For a comprehensive evaluation in case of axial involvement, the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the Bath Ankylosing Spondylitis Functional Index (BASFI) were also assessed in study subjects. With a 0-10 score range and a higher score representing a higher disease activity, the BASDAI consists of six questions pertaining to five major domains (fatigue, spinal pain, joint pain/swelling, areas of localized tenderness, and morning stiffness) [55]. Similarly to BASDAI, the BASFI is based on a 10-cm VAS (0 being no problem and 10 being the worst problem) applied to 10 questions evaluating the patients' ability to cope with activities of daily living, thus addressing the degree of functional limitation in patients with axial involvement (score range: 0-10) [56].

3. Blood laboratory parameters

Venous blood samples were collected to evaluate the following laboratory parameters: total cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, glucose, creatinine, azotemia, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), erythrocyte sedimentation rate (ESR), and CRP.

4. Blood pressure

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the lying position, at 1-min intervals, on three occasions according to European Society of Hypertension-European Society of Cardiology guidelines [57].

5. Brachial artery flow-mediated dilation (FMD) with reactive hyperemia index (RHI)

Patients were asked to abstain from alcohol, tobacco and caffeine for at least 12 hours on the day of examination. Study procedures were performed after overnight fasting and following ≥10 minutes of rest in supine position (a small head pillow was accepted). Brachial artery FMD and reactive hyperemia index (RHI) were evaluated by the same operator blinded to the clinical status of the subjects. FMD and RHI were measured by ultrasound imaging, as described in the guidelines of the International Brachial Artery Reactivity Task Force [32].

FMD and RHI of the brachial artery were evaluated according to a standardized ultrasound protocol using an automatic edge detection software (Cardiovascular Suite®, FMD studio, QUIPU Srl, Pisa, Italy). The examination consisted in measuring brachial artery diameter (BAD) at rest and after reactive hyperemia induced by ischemia of the forearm. The measurement was made on a B-mode section of the artery, which was imaged above the antecubital fossa in the longitudinal plane by using a linear ultrasound vascular transducer with a frequency of 10 MHz (Esaote®, MyLab 25 Gold, Pisa, Italy). Baseline BAD and the flow velocity were recorded for 60 seconds. The blood pressure cuff was placed on the forearm 4-5 cm behind elbow joint line and inflated up to 70 mmHg above the systolic blood pressure to induce a transitory ischemia for 300 seconds. After 5 minutes, the cuff was deflated and the BAD was recorded for 240 seconds after deflation. FMD was calculated as (maximum post-ischemic BAD – basal BAD) / basal BAD x 100 and expressed as percent of BAD increase compared with baseline value. RHI was calculated as (average flow velocity after cuff deflation / flow velocity measured at the baseline) and defined “low” when < 1.67 [58]. The duration of the overall exam was about 10-15 minutes. The reproducibility of this scanning protocol was evaluated on a representative sample of 5 subjects randomly selected from the study population within 1 week from the first examination.

Statistical analysis

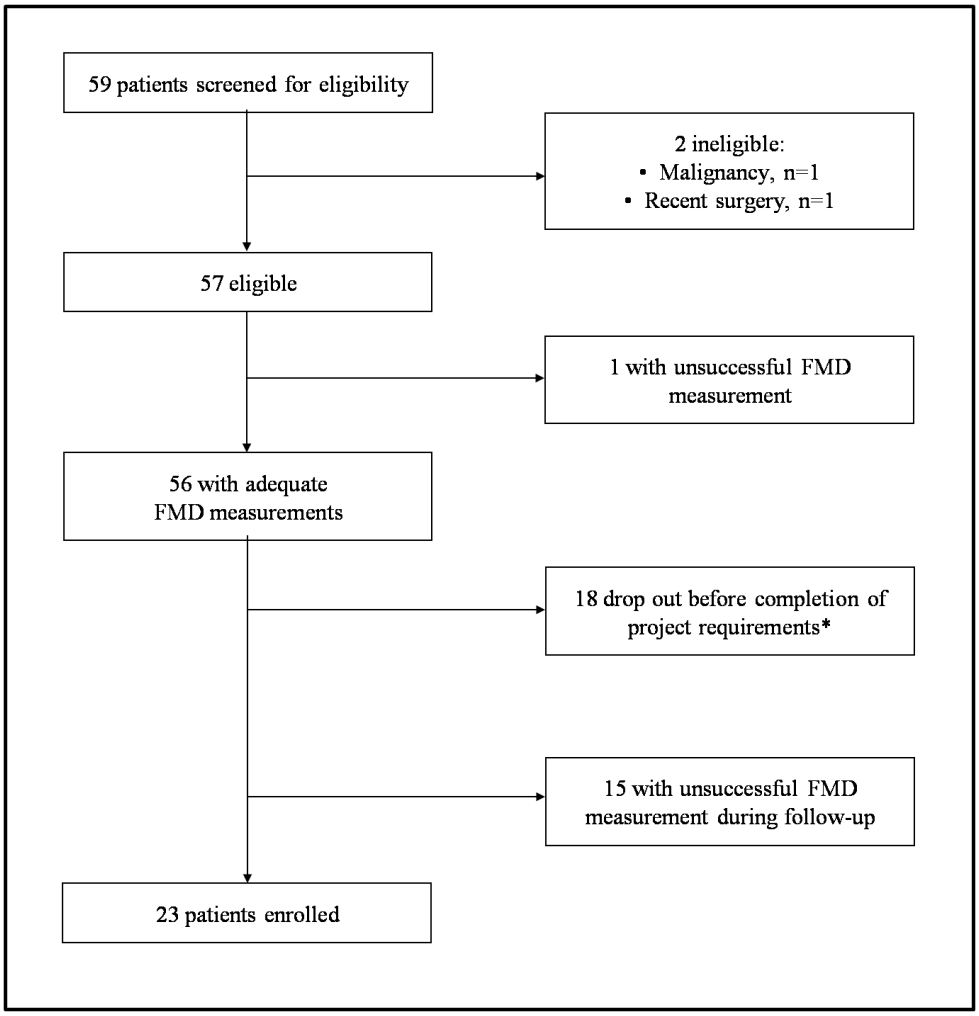
Statistical analysis was performed with the IBM SPSS 22 system (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean ± standard deviation (SD) or as median (interquartile range) in case of skewed distribution. The t-test was performed to compare continuous variables for paired samples and for independent samples. In case of values with a skewed non-Gaussian distribution, Mann-Whitney U test was used to compare means. The χ^2 test or Fisher's exact test were used to compare categorical variables. Relationships between continuous variables and FMD/RHI during the study period were examined using simple regressions with Spearman's rank correlation coefficient (rho). All results were expressed as 2-tailed values, P values <0.05 being statistically significant.

As to sample size, with a pre-defined median increase in FMD from baseline values to $T_{12m} \geq$ 50%, 16 subjects were needed to obtain an 80% power and a 5% α error. Also considering a drop-out risk, at least 20 subjects had to be enrolled in the present study.

4. RESULTS

As reported in Figure 2, of 59 patients screened for eligibility, 2 (3.4%) were ineligible for protocol adherence issues. One (1.7%) out of the 57 eligible PsA patients was excluded because of unsuccessful FMD measurement at baseline. A total of 56 subjects with successful baseline assessment starting apremilast entered the study protocol. Of these, 18 (32.1%) subjects dropped out from the study before completion of project requirements for different reasons. In 15 (26.8%) subjects, FMD measurement during 12-month follow-up failed. Therefore, 23 patients (26.1% males, mean age 60.9 ± 8.6 years) with PsA were included in the final analysis.

Figure 2. Flow chart of study participants.



***9 patients withdrawn informed consent and 9 discontinued treatment with apremilast.**

Table 1. Baseline characteristics of subjects with psoriatic arthritis (PsA) starting a treatment with apremilast.

Variable	PsA patients
Subjects, n	23
Age, years	60.9 ± 8.6
Male gender, n (%)	6 (26.1)
Smoking, n (%)	21 (55.9)
Anthropometric parameters	
Weight, kg	76.6 ± 16.7
Height, cm	164.7 ± 8.6
BMI, kg/m^2	28.3 ± 6.2
Blood laboratory parameters	
Total cholesterol, mg/dl	195.9 ± 39.8
HDL, mg/dl	56.2 ± 15.7
LDL, mg/dl	121.3 ± 38.5
Triglycerides, mg/dl	121.2 ± 56.1
Glucose, mg/dl	103.1 ± 25.8
Creatinine, mg/dl	0.8 ± 0.3
Azotemia, mg/dl	35.9 ± 12.3
Uric acid, mg/dl	4.8 ± 1.2
AST, U/l	18.7 ± 7.8
ALT, U/l	22.5 ± 13.1
ESR, mm/h	27.0 ± 25.3
CRP, mg/dl	9.0 ± 10.9
Rheumatologic parameters	
DAPSA	32.0 ± 15.0
BASDAI	6.7 ± 1.8
BASFI	5.7 ± 2.5
Blood pressure	
SBP, mmHg	128.7 ± 9.2
DBP, mmHg	80.9 ± 5.8
Cardiovascular comorbidities	
Dyslipidemia, n (%)	12 (52.2)
IFG/Diabetes mellitus, n (%)	9 (39.1)
Obesity, n (%)	9 (39.1)
Hypertension, n (%)	15 (65.2)
Heart failure, n (%)	0 (0)
History of myocardial infarction, n (%)	0 (0)
Atrial fibrillation, n (%)	1 (4.3)
History of stroke/TIA, n (%)	1 (4.3)
PAD, n (%)	1 (4.3)
VTE, n (%)	1 (4.3)
Other comorbidities	
Kidney failure, n (%)	2 (8.7)
Chronic hepatic disease, n (%)	1 (4.3)
History of malignancy, n (%)	7 (30.4)

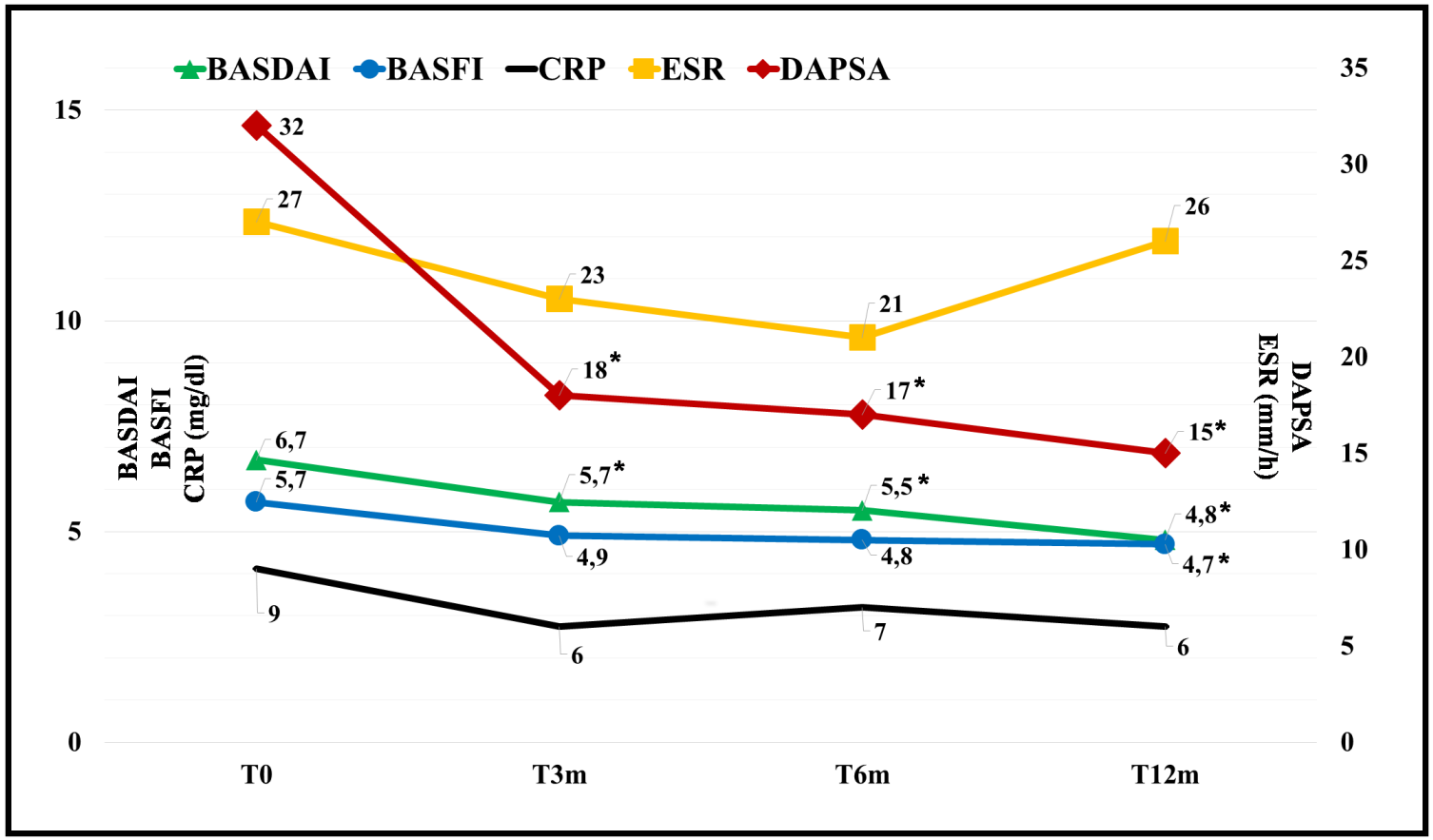
Rheumatic medications	
NSAIDs, n (%)	12 (52.2)
CCS, n (%)	5 (21.7)
MTX, n (%)	4 (17.4)
SFS, n (%)	1 (4.3)
LFN, n (%)	2 (8.7)
Cardiovascular medications	
β-blockers, n (%)	9 (39.1)
CCB, n (%)	7 (30.4)
ACE-I, n (%)	2 (8.7)
ARB, n (%)	11 (47.8)
Statin, n (%)	4 (17.4)
Antiplatelet drugs, n (%)	5 (21.7)
Oral anticoagulants, n (%)	0 (0)
Other medications	
Oral antidiabetic agents, n (%)	5 (21.7)
Insulin, n (%)	3 (13.0)
Allopurinol, n (%)	1 (4.3)

n: number; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ESR: erythrocyte sedimentation rate; CPR: C-reactive protein; DAPSA: Disease Activity Index in Psoriatic Arthritis; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; SBP: systolic blood pressure; DBP: diastolic blood pressure; IFG: impaired fasting glucose; TIA: transient ischemic attack; PAD: peripheral artery disease; VTE: venous thromboembolism; NSAIDs: non-steroidal anti-inflammatory drugs; CCS: corticosteroids; MTX: methotrexate; SFS: sulfasalazine; LFN: leflunomide; CCB: calcium channel blockers; ACE-I: angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blockers. Continuous data are presented as mean ± standard deviation.

Baseline characteristics of the study population are summarized in **Table 1**. The study sample consisted of adult PsA patients with high disease activity (mean DAPSA: 32.0). Patients were generally overweight (mean BMI: 28.3 kg/m^2), with 39.1% of obese subjects. About 50% of them had dyslipidemia, while 65.2% had essential hypertension. However, considering the high percentage of subjects using CV medications, blood pressure values were generally normal. Diabetes mellitus was documented in 39.1% of patients and smoking habit in 55.9%. The majority of patients used NSAIDs at study entry.

The effect of apremilast on main clinical and laboratory measures of disease activity and function during the overall study period are summarized in **Figure 3**.

Figure 3. Changes in main clinical and laboratory measures of disease activity and function during 12-month treatment with apremilast.

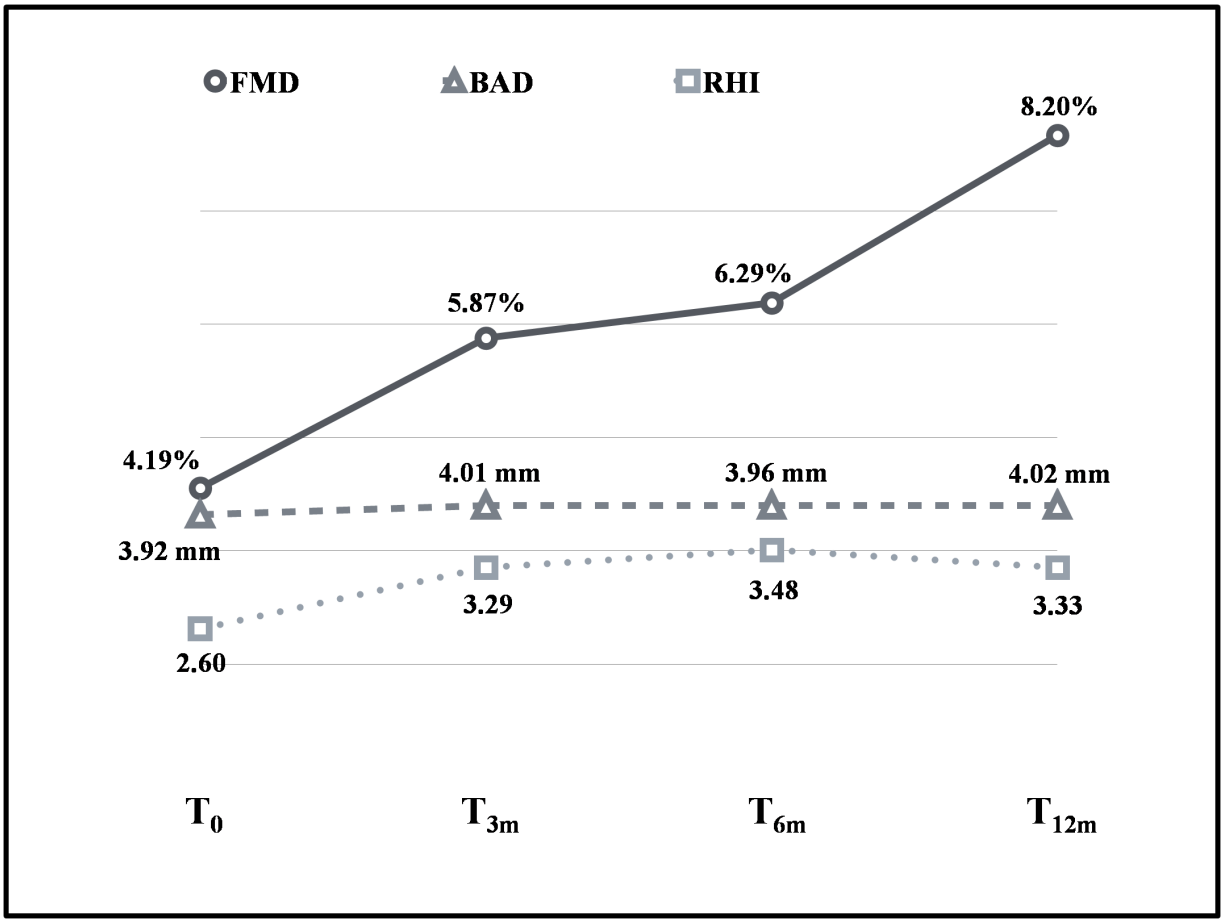


DAPSA: Disease Activity Index in Psoriatic Arthritis; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; CPR: C-reactive protein; ESR: erythrocyte sedimentation rate; T_0: baseline; T_{3m}: 3 months after starting treatment with apremilast; T_{6m}: 6 months after starting treatment with apremilast; T_{12m}: 12 months after starting treatment with apremilast.

*P *vs.* baseline <0.05

As compared to baseline values, a significant reduction in DAPSA and BASDAI scores was found at each time-point (P always <0.05), with a progressive decrease in both parameters during the 12-month follow-up and an overall 56.6% and 28.7% median reduction from T_0 to T_{12m}, respectively. At each time-point, a progressive improvement of BASFI values was also documented, with an overall 33.3% median reduction from T_0 to T_{12m} (P=0.012). In contrast, non-significant changes in CRP and ESR levels were documented during the study period.

Figure 4. Changes in brachial artery diameter (BAD), flow-mediated dilation (FMD) and reactive hyperemia index (RHI) during 12-month treatment with apremilast.



T_0: baseline; T_{3m}: 3 months after starting treatment with apremilast; T_{6m}: 6 months after starting treatment with apremilast; T_{12m}: 12 months after starting treatment with apremilast.

At baseline assessment (Figure 4), the 23 PsA patients showed a BAD of 3.92 ± 0.43 mm, FMD of 4.19% ± 2.35 and RHI of 2.60 ± 0.59.

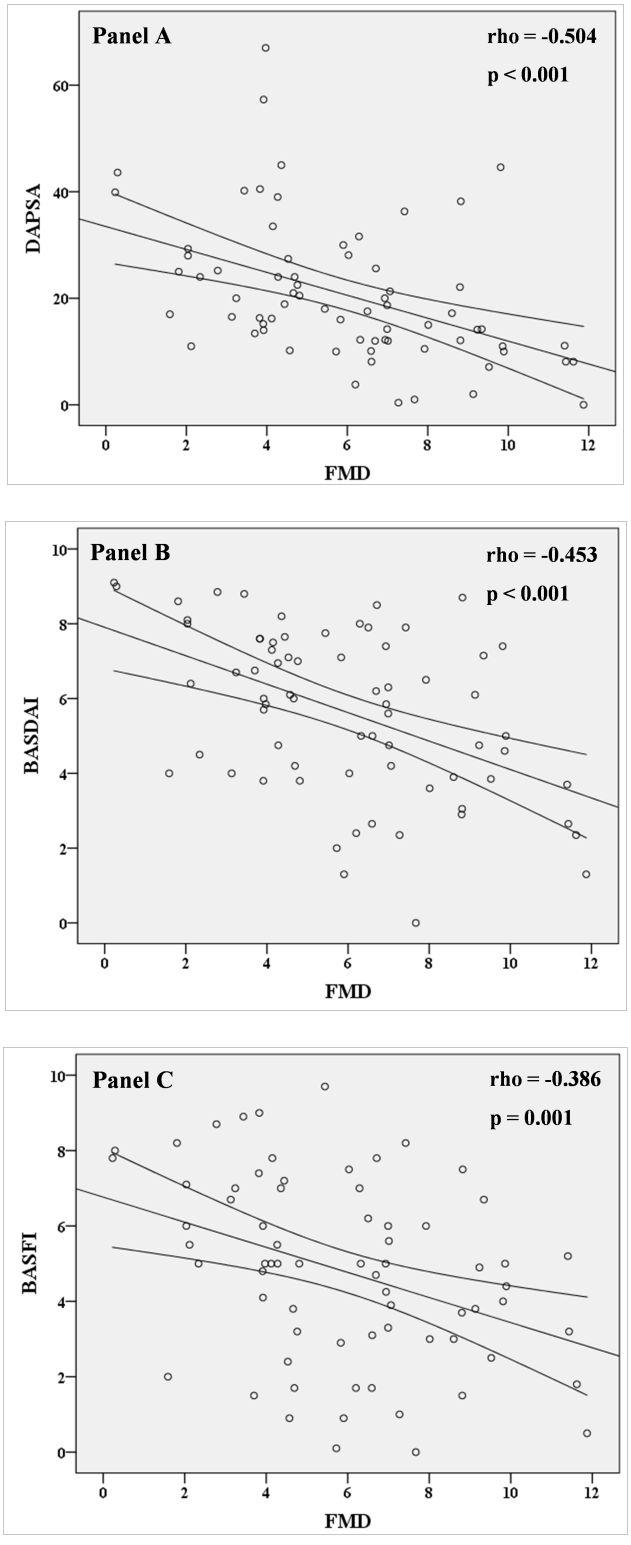
After 3 months of treatment with apremilast (T_{3m}), no significant change in BAD occurred (from 3.92 ± 0.43 mm to 4.01 ± 0.57 mm, P=0.329). FMD changed from 4.19% ± 2.35 to 5.87% ± 2.09 (P=0.006), corresponding to a 41.1% median increase, with a 17.0% median increase in RHI values (from 2.60 ± 0.59 to 3.29 ± 0.83, P=0.027).

At T_{6m}, BAD was similar to baseline values (from 3.92 ± 0.43 mm to 3.96 ± 0.69 mm, P=0.488), while FMD showed a 53.9% median increase as compared to T_0 (from 4.19% ± 2.35 to 6.29% ± 2.76, P=0.005). RHI changed from 2.60 ± 0.59 to 3.48 ± 1.74 (P=0.016), corresponding to a 33.2% median increase.

As compared to baseline values, BAD was substantially unchanged (4.02 ± 0.68 mm, P=0.236) at the end of the follow-up period (T_{12m}). A 90.4% median increase in FMD was reported at T_{12m} (from 4.19% ± 2.35 to 8.20% ± 2.30, P<0.001), with a corresponding 24.2% median increase in RHI values (from 2.60 ± 0.59 to 3.33 ± 0.99, P=0.007).

Overtime values of FMD showed an inverse correlation with DAPSA (rho=-0.504; P<0.001, **Figure 5A**), BASDAI (rho=-0.453; P<0.001, **Figure 5B**), and BASFI (rho=-0.386; P=0.001, **Figure 5C**). A direct correlation between overtime FMD and RHI values was also documented (rho=0.406; P<0.001), thus suggesting consistent changes in vascular distensibility and reactive hyperemia.

Figure 5. Relationship between overtime values of FMD and overtime values of DAPSA (Panel A), BASDAI (Panel B), and BASFI (Panel C) during 12-month treatment with apremilast.



FMD: flow-mediated dilation; DAPSA: Disease Activity Index in Psoriatic Arthritis; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index.

5. DISCUSSION

In the present prospective study, we documented a significant improvement in endothelial function of PsA patients during a 12-month treatment with apremilast. In detail, we found a progressive and significant improvement of FMD values at each time-point, with an overall 90.4% median increase from baseline to 12 months (from 4.19% to 8.20%) and a corresponding 24.2% median increase in RHI values (from 2.60 to 3.33).

Overall, our results are in line with a great amount of literature data supporting the hypothesis that inflammatory suppression may somehow impact CV risk of patients with rheumatic diseases [37]. Chronic inflammation may represent the common pathogenic substrate of PsA and atherosclerosis, thus it can be hypothesized that controlling inflammation may somehow provide CV benefits to patients with PsA. According to DAPSA score, our study population consisted of patients with high disease activity (DAPSA >28) at study entry, with two patients achieving complete remission (DAPSA ≤4) at 6-month follow-up and three after 12 months. However, apremilast determined a progressive and significant improvement of clinical measures of disease activity and function during the overall study period, with a consistent reduction of DAPSA, BASDAI and BASFI and an inverse correlation between overtime values of FMD and these clinical parameters. This suggests a correlation between the improvement of vascular reactivity and the reduction of the inflammatory state in our patients.

In previous studies, the use of traditional DMARDs and biologic agents showed promising but, sometimes, contrasting results in terms of CV risk reduction in patients with rheumatic diseases [37]. In line with results of our study, methotrexate showed that its favorable effect on CV outcome may depend on clinical response, since a lower incidence of myocardial infarction was observed in responders to therapy [39]. A reduced incidence of major CV events (stroke, myocardial infarction and CV death) [40], with a significant improvement of

arterial stiffness [45] and endothelial function [43,44] has been associated to the use of TNFα-blockers, anti-IL-6 or other biologic agents commonly used for the treatment of rheumatoid arthritis. Overall, most evidence on the impact of anti-inflammatory therapies on the atherosclerotic burden of rheumatic diseases come from patients with rheumatoid arthritis, while little is known about the CV consequences of controlling inflammation in subjects with PsA. To the best of our knowledge, this is the first study documenting a significant and progressive increase of FMD accompanied by a parallel improvement in RHI in PsA patients during a 12-month therapy with a novel targeted synthetic DMARD.

Our results are of particular clinical interest considering that, based on meta-analytical data, patients with PsA display impaired endothelial function, as expressed by lower FMD values [12], and that each 1% decrease in FMD values is associated with a 12% increase of CV events [59]. Several mechanisms have been proposed to justify such high atherosclerotic burden in this clinical setting [13]. Although PsA patients exhibit an increased prevalence of diabetes, hypertension, hyperlipidaemia and obesity [11], the relationship between subclinical atherosclerosis and PsA seems to be more complex and the prevalence of these traditional VRFs might not entirely explain the accelerated atherosclerotic process in these patients. At this regard, immune-mediated inflammation might represent the link between PsA and atherosclerosis, with a number of pro-inflammatory cytokines (TNFα, IL-1, IL-6, IL-17) [14] and acute-phase proteins (CRP, fibrinogen) [15] being involved in this process. As a consequence, PsA patients have a high risk of early carotid atherosclerosis, a higher plaque instability and a pro-coagulant state [12], thus leading to a higher incidence of ischemic events and making CV mortality one of the leading cause of death in this clinical setting [5,11]. We do not have evidence about all mechanisms underlying the improvement in endothelial function following treatment with apremilast, but it can be hypothesized that the decrease in plasma levels of pro-inflammatory cytokines might influence vascular reactivity

via several mechanisms, such as an increased NO production. In rheumatic diseases, reduced bioavailability of NO has been suspected to play a pathogenic role in endothelial dysfunction [23]. In animal models, PDE4 inhibition has shown to increase NO production by modulating dimethylarginine dimethylaminohydrolases (DDAH) expression [52]. NO is synthesized by endothelial NO synthase (eNOS), being a potent vasodilator and playing an important role in regulating vascular tone [23]. Impairment of eNOS activity by endogenous eNOS inhibitors, such as ADMA, has been implicated in endothelial dysfunction [23]. DDAH is the ADMA-metabolizing enzyme and PDE4 inhibition has shown to up-regulate its expression. Large prospective studies are needed to confirm and to better understand the potential mechanisms on the basis of our results.

Some potential limitations of the present study need to be discussed.

First, we have to consider that vascular reactivity evaluation might be biased by operator dependent variability. However, in the present study we evaluated vascular reactivity by means of FMD, a non-invasive validated technique [60], known to be related to the prevalence and extent of coronary atherosclerosis [61], and to be able to predict long-term CV risk [62]. To reduce variability and to optimize repeatability of results, we used a Food and Drug Administration (FDA) approved software allowing for an automated diameter and flow assessment.

A potential confounding effect could be also related to differences in BAD. Some previous data showed that changes in BAD could impact on FMD [63]. Interestingly, in the present study we tested BAD overtime and confirmed no significant changes during the study period. Thus, all reported changes in FMD are likely secondary to modifications in vascular reactivity.

A further limitation of our study is that most of patients included in the present study had concomitant CV risk factors, potentially impacting on vascular reactivity. To evaluate

potential sources of heterogeneity, we should have performed a sensitivity analysis after stratifying patients according to major clinical and demographic characteristics. However, the relatively low number of participants in our study did not allow to perform any sensitivity analysis.

Finally, the lack of a control group might represent another limitation of the present study. However, the pre-post observational design allowed for partly overcome the intrinsic inter-individual heterogeneity, with each patient being the control of himself.

6. CONCLUSIONS

In line with a great amount of literature data suggesting the presence of an increased atherosclerotic burden in patients with PsA, our results suggest the potential role of apremilast and anti-inflammatory therapies in improving endothelial function, thus potentially modifying CV risk in this clinical setting. The correlation between the improvement of vascular reactivity and the reduction of disease activity in our patients further confirms that inflammation might represent the link between PsA and accelerated atherosclerosis.

Patients with PsA may benefit from a periodic assessment of surrogate markers of CV risk, such as FMD with RHI. This could help to establish combined and more specific prevention and therapeutic strategies aimed to reduce both disease activity and CV risk in this clinical setting.

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