Severity of Psoriasis Associates With Aortic Vascular Inflammation Detected by FDG PET/CT and Neutrophil Activation in a Prospective Observational Study

Haley B. Naik, Balaji Natarajan, Elena Stansky, Mark A. Ahlman, Heather Teague, Taufiq Salahuddin, Qimin Ng, Aditya A. Joshi, Parasram Krishnamoorthy, Jenny Dave, Shawn M. Rose, Julia Doveikis, Martin P. Playford, Ronald B. Prussick, Alison Ehrlich, Mariana J. Kaplan, Benjamin N. Lockshin, Joel M. Gelfand, Nehal N. Mehta

Objective—To understand whether directly measured psoriasis severity is associated with vascular inflammation assessed by 18F-fluorodeoxyglucose positron emission tomography computed tomography.

Approach—In-depth cardiovascular and metabolic phenotyping was performed in adult psoriasis patients (n=60) and controls (n=20). Psoriasis severity was measured using psoriasis area severity index. Vascular inflammation was measured using average aortic target-to-background ratio using 18F-fluorodeoxyglucose positron emission tomography computed tomography.

Results—Both the psoriasis patients (28 men and 32 women, mean age 47 years) and controls (13 men and 7 women, mean age 41 years) were young with low cardiovascular risk. Psoriasis area severity index scores (median 5.4; interquartile range 2.8–8.3) were consistent with mild-to-moderate skin disease severity. Increasing psoriasis area severity index score was associated with an increase in aortic target-to-background ratio (β=0.41; P=0.001), an association that changed little after adjustment for age, sex, and Framingham risk score. We observed evidence of increased neutrophil frequency (mean psoriasis, 3.7±1.2 versus 2.9±1.2; P=0.02) and activation by lower neutrophil surface CD16 and CD62L in blood. Serum levels of S100A8/A9 (745.1±53.3 versus 195.4±157.8 ng/mL; P<0.01) and neutrophil elastase-1 (43.0±2.4 versus 30.8±6.7 ng/mL; P<0.001) were elevated in psoriasis. Finally, S100A8/A9 protein was related to both psoriasis skin disease severity (β=0.53; P=0.02) and vascular inflammation (β=0.48; P=0.02).

Conclusions—Psoriasis severity is associated with vascular inflammation beyond cardiovascular risk factors. Psoriasis increased neutrophil activation and neutrophil markers, and S100A8/A9 was related to both skin disease severity and vascular inflammation. (Arterioscler Thromb Vasc Biol. 2015;35:35-00. DOI: 10.1161/ATVBHA.115.306460.)

Key Words: inflammation ■ neutrophils ■ PET ■ CT ■ psoriasis ■ vascular inflammation

Psoriasis is a chronic inflammatory disease that affects 2% to 3% of the adult population1 and is associated with atherosclerosis.2 Emerging evidence suggests that psoriasis may be an independent risk factor for life-threatening complications of atherosclerosis, including myocardial infarction, stroke, and premature cardiovascular death.2–8 Severe psoriasis is associated with a 58% increased risk of major adverse cardiac events and a 57% increased risk of cardiovascular death, and dose-response evidence for myocardial infarction suggests that the severity of psoriatic skin disease may be associated with the degree of systemic inflammation and extent of cardiovascular disease.5,7,9 Despite these data, however, cardiovascular disease may be underdiagnosed and undertreated in psoriasis,10 and in vivo data on the relationship between psoriasis severity and vascular diseases are sparse.11,12

Recent studies from our group and others demonstrate increased vascular inflammation in patients with psoriasis as compared with age-matched healthy controls, measured by 18F-fluorodeoxyglucose positron emission tomography computed tomography (FDG PET/CT).11–14 These were pilot studies, which provided important proof of concept for use of FDG in detecting inflammation in psoriatic diseases. These pilot studies only included severe psoriasis patients and performed limited cardiometabolic testing. Furthermore, small
sample sizes did not permit stratified analyses or adjustment for multiple potential confounders. FDG uptake in the blood vessel wall by PET/CT is associated with activated endothelial cells and cellular infiltration in noncalcified, atherosclerotic plaques. Prior studies demonstrate that FDG in the vessel wall is taken up by macrophages, which are CD68+ cells critical in atherogenesis. Furthermore, recent investigations have demonstrated that FDG uptake directly associates with vascular inflammation and reliably detects changes in atherosclerotic plaques in at-risk populations, including individuals with rheumatoid arthritis and those infected with HIV. Notably, vascular uptake of FDG is associated with future cardiovascular events and improves with lifestyle modification and statin therapy. These findings demonstrate that FDG PET/CT provides a reliable and reproducible measure to characterize the relationship between psoriasis severity and vascular inflammation.

There are differential gene expression signatures in psoriatic skin plaques with associated changes in protein levels of these same genes in the blood associated with cardiovascular diseases. Furthermore, accumulating evidence suggests that shared biological pathways between psoriasis and cardiometabolic diseases may provide a plausible link between psoriasis and vascular diseases. Moreover, large population-based studies demonstrate that body surface area affected by psoriasis directly correlates with cardiometabolic diseases. Thus, we hypothesized that psoriatic skin disease severity quantified by the psoriasis area severity index (PASI) score would be associated with vascular inflammation measured by FDG PET/CT.

Neutrophils are the earliest cells present in psoriatic plaques, and recent data indicate that neutrophils may be one of the predominant cellular sources of proinflammatory cytokine interleukin (IL)-17 in psoriatic skin disease. Studies also suggest that there may be an important role for peripheral blood neutrophils in psoriasis comorbidities, including coronary artery disease. Therefore, proteins associated with neutrophils, such as myeloperoxidase (MPO), S100A8/A9, and neutrophil elastase-1 (NE-1), may further provide a link between psoriasis and cardiometabolic diseases. Therefore, our objective in this study was to characterize the relationship between directly measured psoriatic skin disease and vascular inflammation and to perform studies aimed at exploring the biological link between these 2 disease entities by characterizing neutrophil frequency, activation, and proteins in psoriasis.

Materials and Methods

Complete details of Materials and Methods, including participant selection, clinical assessments, imaging procedures and analyses, and translational studies, are available in the online-only Data Supplement.

Results

Study Population

Recruitment scheme (Figure 1) and characteristics of the psoriasis (n=60) and the age- and sex-matched control (n=20) study populations are presented (Table 1). Participants were young (median age: psoriasis 47.5 years, interquartile range [IQR] 38.0–54.0; controls 41.0 years, IQR 27.2–52.0) and overweight (mean body mass index psoriasis 29.4, SD 5.7; controls 27.4, SD 5.5). About half of the participants had a past medical history of dyslipidemia (psoriasis n=32, 53%; controls n=10, 50%). We observed mild insulin resistance in psoriasis (median homeostatic model assessment–insulin resistance psoriasis 2.9, IQR 2.0–4.8; controls 2.4, IQR

Recruitment Scheme
1.5–3.5), although the prevalence of diabetes mellitus was low (psoriasis n=7, 12%; controls n=1, 5%) and fasting blood glucose was near normal (median psoriasis 94.0, IQR 88.0–102.5; controls 93.0, IQR 87.5–105.0 mg/dL). Median PASI score in the psoriasis cohort was 5.4 (IQR 2.8–8.3), consistent with mild-to-moderate psoriasis. Average psoriasis disease duration in this cohort was 19.5 years (IQR 5.0–30.5). About half of the participants were on systemic therapy for psoriasis management (40% on biological therapy and 8% on any other systemic agent). Neutrophil:lymphocyte ratio was slightly elevated in the psoriasis cohort (mean 2.1, SD 0.8), and high-sensitivity C-reactive protein levels were elevated (median 2.0, IQR 0.9–4.4 mg/L), suggesting subclinical systemic inflammation. Overall, both psoriasis and control groups were at low risk for cardiovascular disease by Framingham risk score (FRS; median psoriasis 3, IQR 1–6; controls 1, IQR 1–3.5). Arterial inflammation was increased in psoriasis patients compared with controls (target-to-background ratio [TBR] 1.8 versus 1.6; P<0.01). No sex-based or racial/ethnic-based differences were present in either the psoriasis or control cohorts with relationship to vascular inflammation.

**Psoriasis Severity and Cardiometabolic Risk Factors Are Associated With Vascular Inflammation**

In psoriasis patients, univariate analyses demonstrated a positive association between PASI score and aortic TBR (β=0.41, P=0.001), suggesting a relationship between psoriasis and vascular inflammation (Table II in the online-only Data Supplement). Additionally, aortic TBR was positively associated with other known cardiovascular disease risk factors, including body mass index (β=0.51, P<0.001), systolic blood pressure (β=0.43, P=0.001), hyperlipidemia (β=0.26, P=0.04), and type 2 diabetes mellitus (β=0.41, P=0.001).
current tobacco use ($\beta=0.30, P=0.02$), homeostatic model assessment–insulin resistance ($\beta=0.42, P=0.001$), and FRS ($\beta=0.40, P=0.002$). Aortic TBR had a negative association with high-density lipoprotein cholesterol ($\beta=-0.25, P=0.05$).

Psoriasis Severity Is Associated With Vascular Inflammation Independent of Cardiovascular Risk Factors

In unadjusted linear regression models, increasing PASI score was associated with an increase in vascular inflammation as measured by aortic TBR ($\beta=0.41, P=0.001$). This relationship remained significant after adjusting for age and sex ($\beta=0.39, P=0.002$) and FRS ($\beta=0.39, P=0.001$; Table 2 and Figure 2A and 2B).

Psoriasis Severity Predicts Vascular Inflammation Beyond FRS and High-Sensitivity C-Reactive Protein in Fully Adjusted Models

The contribution of PASI score in predicting vascular inflammation (aortic TBR) beyond FRS and high-sensitivity C-reactive protein was determined using likelihood ratio testing in nested models (Table 3). PASI score added incremental value beyond FRS ($\chi^2=8.66, P<0.01$) and high-sensitivity C-reactive protein ($\chi^2=6.48, P=0.01$) when added to nested models, suggesting that psoriatic skin disease severity independently increases vascular inflammation.

Cytokines Associated With Innate Immune Activation and Cytokines That Modulate Neutrophil Biology Are Elevated in Psoriasis Plasma

Markers of increased innate immune activation, including IL-6 (mean psoriasis 1.58±0.37 versus controls 0.56±0.06 pg/mL; $P=0.044$) and tumor necrosis factor–α (mean psoriasis 7.16±2.49 versus controls 1.53±0.09 pg/mL; $P=0.002$), were increased in psoriasis patients (Figure 3). We then hypothesized that skin disease severity may be associated with vascular inflammation via neutrophils. Consistent with this, we

Table 2. Multivariable Adjusted Linear Regression of Psoriasis Severity With Aortic Vascular Inflammation

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>0.41 (0.001)</td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>0.39 (0.002)</td>
</tr>
<tr>
<td>Adjusted for Framingham risk score</td>
<td>0.39 (0.001)</td>
</tr>
</tbody>
</table>

Data on table represented as standardized regression coefficient (P value).
observed increased levels of cytokines associated with neutrophil recruitment, differentiation, activation, and release; granulocyte macrophage colony-stimulating factor (GM-CSF; mean psoriasis 0.34±0.03 versus controls 0.23±0.04 pg/mL; P=0.049) and IL-17 (mean psoriasis 3.51±0.84 versus controls 1.83±0.51 pg/mL; P=0.05) were elevated in psoriasis plasma compared with that of controls (Figure 3).

Neutrophil Frequencies, Activation, and Markers Are Elevated in Psoriasis

After observing that absolute neutrophil count was increased in psoriatic individuals when compared with the controls (psoriasis mean 3.7±1.2 versus controls mean 2.9±1.2; P=0.02), immunophenotyping by flow cytometry confirmed a significant increase in neutrophil frequencies in the circulating whole blood (psoriasis, mean 56.3±13.8; controls, mean 56.3±13.8; P<0.01; Figure 4A and 4B; Figure II in the online-only Data Supplement). The mean fluorescence intensity of CD16 and CD62L on the neutrophil surface, 2 membrane-bound molecules shown to be enzymatically cleaved from activated leukocytes, were decreased in psoriasis patients, though CD62L did not reach statistical significance (CD62L: psoriasis mean 245.3±85.2, controls mean 248.3±64.79; P=0.07; and CD16: psoriasis mean 18185±8525, controls mean 24285±6479; P<0.01; Figure 4C). Subsequent analysis

Table 3. Relative Value of PASI, Framingham Risk Score, and High-Sensitivity C-Reactive Protein in Predicting Vascular Inflammation

<table>
<thead>
<tr>
<th>Variable</th>
<th>χ²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASI added to model*</td>
<td>7.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FRS added to model*</td>
<td>0.89</td>
<td>0.34</td>
</tr>
<tr>
<td>hsCRP added to model*</td>
<td>0.71</td>
<td>0.10</td>
</tr>
<tr>
<td>PASI added to FRS in model*</td>
<td>8.66</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FRS added to PASI in model*</td>
<td>2.45</td>
<td>0.12</td>
</tr>
<tr>
<td>PASI added to hsCRP in model*</td>
<td>6.48</td>
<td>0.001</td>
</tr>
<tr>
<td>hsCRP added to PASI in model*</td>
<td>0.10</td>
<td>0.76</td>
</tr>
</tbody>
</table>

FRS indicates Framingham LDL 10-year risk score; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; and PASI, psoriasis area and severity index. Likelihood ratio testing was applied to nested Tobit models to assess the incremental value of PASI, FRS, and hsCRP, and vice versa, in predicting vascular inflammation.

*pAll models included age, sex, systolic blood pressure, fasting glucose, low-density lipoprotein, and current tobacco use.
Neutrophils Relate to Skin Disease and S100A8/A9 Relates to Both Skin and Vascular Disease

We examined whether absolute neutrophil count related to psoriasis severity and found that absolute neutrophil count was related to PASI score ($\beta=0.24; P=0.03$). Finally, we tested whether these neutrophil markers were related to PASI score (Table 4) and vascular inflammation (Table 5). Neither MPO ($\beta=-0.06; P=0.82$) nor NE-1 ($\beta=0.16; P=0.47$) related to PASI score in fully adjusted models. However, S100A8/A9 ($\beta=0.53; P=0.02$) showed a relationship with psoriasis severity beyond adjustment for age and sex. Similarly, neither MPO ($\beta=-0.20; P=0.32$) nor NE-1 ($\beta=0.08; P=0.49$) related to vascular inflammation, but a relationship was observed between S100A8/A9 and vascular inflammation when adjusted for FRS ($\beta=0.48; P=0.02$; Table 5 and Figure 6). These findings suggest that this S100A8/A9 protein may serve as a mediator or by-product in one of the potential pathways linking psoriasis severity and arterial inflammation.

Discussion

In this well-characterized cohort of patients with psoriasis, we demonstrate 6 principal findings: (1) metabolic derangement and subclinical systemic inflammation in psoriasis; (2) psoriasis severity, assessed by PASI score, was associated with aortic vascular inflammation, as measured by aortic TBR, beyond cardiovascular risk factors; (3) psoriasis severity predicted vascular inflammation beyond FRS and high-sensitivity C-reactive protein; (4) cytokines associated with innate immune activation were increased in psoriasis blood; (5) neutrophil frequency, activation, chemoattractant cytokines, and protein markers were elevated in psoriasis blood; and (6) S100A8/A9 was related to both skin disease severity and vascular inflammation beyond the FRS.

Several studies demonstrate that psoriasis patients are at increased risk of cardiovascular diseases. Moreover, severe psoriasis is independently associated with atherosclerotic cardiovascular disease. Recent studies further demonstrate that the risk of cardiovascular events, cardiovascular death, and reduced life expectancy because of cardiovascular death are increased in severe psoriasis, particularly younger individuals, and is comparable to that seen in diabetics. Although some of the cardiovascular risk seen in this patient population may be related to the increased rates of obesity, hypertension, diabetes mellitus, hyperlipidemia, metabolic syndrome, and smoking observed in psoriasis patients, the chronic systemic inflammation associated with skin inflammation in psoriasis likely contributes to this risk.

FDG is a reliable and reproducible biomarker for atherosclerosis in at-risk populations, allowing for noninvasive measurement of vessel wall inflammation. Vascular uptake of FDG represents cells important in atherosclerosis. FDG uptake in the vasculature associates with cardiovascular disease biomarkers and unstable atherosclerotic plaques, predicts vascular calcifications, detects changes in atherosclerotic plaque inflammation in participants with cardiovascular disease or with high risk of cardiovascular disease, and reduces with treatment for cardiovascular disease risk factors in animal models and humans with lifestyle modification and statin therapy. Furthermore, a recent investigation in HIV patients demonstrated a relationship between arterial inflammation detected by FDG PET/CT and high-risk coronary atherosclerotic plaque, which are predominantly made up of cells present in psoriatic plaques. Similarly, increased FDG vascular uptake is shown in other inflammatory conditions associated with higher cardiovascular disease risk, including rheumatoid arthritis. FDG PET/CT can detect vascular inflammation in the setting of psoriasis, and we observed increased inflammation in severe psoriasis as compared with mild psoriasis and the general population. These data indicate

Table 4. Regression Analysis of Neutrophil Markers With Psoriasis Area and Severity Index (PASI) Score

<table>
<thead>
<tr>
<th>ELISA Protein</th>
<th>Unadjusted</th>
<th>Model 1*</th>
<th>Model 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloperoxidase, ng/mL</td>
<td>−0.16 (0.39)</td>
<td>−0.16 (0.42)</td>
<td>−0.06 (0.82)</td>
</tr>
<tr>
<td>S100A8/A9, ng/mL</td>
<td>0.42 (0.02)</td>
<td>0.45 (0.03)</td>
<td>0.53 (0.02)</td>
</tr>
<tr>
<td>Neutrophil elastase-1, ng/mL</td>
<td>−0.16 (0.41)</td>
<td>−0.15 (0.45)</td>
<td>−0.16 (0.47)</td>
</tr>
</tbody>
</table>

Data on the table expressed as standardized regression coefficient ($P$ value).

*Adjusted for age and sex.

†Adjusted for Framingham risk score.
that FDG PET/CT serves as a tool to examine vascular inflammation in patients with psoriasis.

The association between severity of skin disease and vascular inflammation found in our study suggests a potentially shared immune-mediated mechanism. Traditionally, this was deemed to be T lymphocyte-mediated, with some contributions from dendritic cells and monocytes in the setting of proinflammatory cytokines. Inflammatory mediators produced by these cells in psoriatic plaques circulate systemically, thereby affecting various tissues, including vascular endothelium, and leading to vascular inflammation and atherosclerotic disease. In support of this model, transcriptome analysis demonstrated that cytokines and chemokines upregulated in psoriasis are also increased in atherosclerosis.

Table 5. Regression Analysis of Neutrophil Protein Markers With Vascular Inflammation

<table>
<thead>
<tr>
<th>ELISA Protein</th>
<th>Unadjusted</th>
<th>Model 1*</th>
<th>Model 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloperoxidase, ng/mL</td>
<td>−0.17 (0.37)</td>
<td>−0.18 (0.38)</td>
<td>−0.20 (0.32)</td>
</tr>
<tr>
<td>S100A8/A9, ng/mL</td>
<td>0.55 (0.001)</td>
<td>0.54 (&lt;0.01)</td>
<td>0.48 (0.02)</td>
</tr>
<tr>
<td>Neutrophil elastase-1, ng/mL</td>
<td>0.06 (0.63)</td>
<td>0.02 (0.89)</td>
<td>0.08 (0.49)</td>
</tr>
</tbody>
</table>

Data on the table expressed as standardized regression coefficient (P-value).

*Adjusted for age and sex.
†Adjusted for Framingham risk score.

Figure 6. Circulating S100A8/A9 levels are associated with psoriasis severity and vascular inflammation by 18F-fluorodeoxyglucose positron emission tomography computed tomography (FDG PET/CT). A, Regression plots for multivariable regression analysis of S100A8/A9 with psoriasis area and severity index (PASI) score for untreated psoriasis patients. B, Regression plots for multivariable regression analysis of S100A8/A9 with vascular inflammation as measured by target-to-background ratio (TBR). CI indicates confidence interval.
including IL-8, IL-36, and chemokine ligand-2.33 In our study, we observed elevated psoriasis biomarkers, tumor necrosis factor-α, and IL-6, which also contribute to the development of atherosclerosis,39 although IL-6 was not significant after correction for multiple testing.

The role of neutrophils in psoriasis is recently gaining interest given the success of anti-IL-17 therapy.40 In addition to being the earliest cell types present in psoriatic plaques41 and one of the predominant cellular sources of proinflammatory cytokine IL-17,42 neutrophils also play an important role in psoriasis comorbidities, such as atherosclerotic diseases.30,32,44 These data suggest that neutrophils may play a role in perpetuating psoriasis and its comorbidities.29,31,45 Interestingly, the cocktail of T-lymphocyte-derived cytokines, IL-17A, tumor necrosis factor-α, and GM-CSF, that effect neutrophil biology were elevated in psoriasis in our study,31 although IL-17A and GM-CSF were not significant after correction for multiple testing. We also found an increased frequency of circulating neutrophils in psoriasis subjects, concomitant with an activated neutrophil phenotype evident by increased shedding of the Fc receptor CD1660 and CD62L, the L-selectin responsible for the tethering and rolling of leukocytes to the endothelium.52 The downregulation of CD16 and CD62L on psoriatic neutrophils suggests an impaired ability to mediate inflammatory damage and a heightened state of activation. This may affect effector functions of neutrophils in vivo and adversely influence the capacity of endothelial cells to support subsequent interactions with circulating leukocytes. Further, neutrophil markers, including serum MPO, S100A8/A9, and NE-1 levels, in our psoriasis cohort were significantly higher than in patients without psoriasis, although MPO weakly met statistical significance after correction for multiple testing. Neutrophil MPO is thought to play a role in initiation, propagation, and acute complications of atherosclerosis,44 and NE-1 is a serine protease which has been shown to be increased at sites of thrombosis and thus has been implicated in the pathogenesis of acute coronary syndrome.48 S100A8/A9 (MRP8/14) is a heterodimeric protein complex synthesized by activated neutrophils and abundant in various inflammatory diseases, including psoriasis, where levels are upregulated in lesional skin.50 Of note, although S100A8/A9 plays an important role in increasing circulating neutrophils and may thereby contribute to cardiovascular disease pathogenesis,51,52 it is not a neutrophil-specific marker. In our study, we demonstrated that S100A8/A9 was associated with PASI score. Furthermore, S100A8/A9 was also related strongly to vascular inflammation, suggesting that neutrophil activity may provide important biological links between vascular inflammation and psoriasis. Our observation that the psoriatic atherosclerotic milieu is upregulated in concert with markers of neutrophil activation underscores the concept that atherosclerosis may involve complex neutrophil biology, which future studies should focus on elucidating, especially after intervention.

To our knowledge, this study is the first attempt at systematic characterization of psoriasis severity and aortic vascular inflammation. Our study is limited by the cross-sectional design and inability to prove causality. Ongoing studies of treatment effect (NCT01553058) and outcomes (NCT017778569) aim to elucidate these relationships. We acknowledge that FDG is a surrogate marker for vascular inflammation. However, ongoing studies with directly quantified coronary artery disease using coronary CT angiography concurrently with FDG PET/CT are currently underway (NCT01778569), which will elucidate the accuracy of FDG PET/CT as a marker of coronary artery disease. We also acknowledge that the protein measurements, including cytokines and chemokines, tested in this study, including IL-6, IL-17, and GM-CSF, did not meet statistical significance after Bonferroni correction for multiple testing.

Conclusions

In conclusion, this study provides strong evidence that psoriasis severity is linked with increased vascular inflammation beyond risk factors for vascular disease. Furthermore, psoriasis severity predicted vascular inflammation beyond the FRS, suggesting that psoriasis plaques are biologically active in promoting inflammation at remote sites. Finally, we demonstrate evidence of increased neutrophil activation and neutrophil markers and that S100A8/A9 is related to both skin disease and vascular inflammation, suggesting an important role of neutrophils in psoriasis-associated cardiovascular disease.

Acknowledgements

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Disclosures

None.

References

Psoriasis Severe and Vascular Inflammation


This study is the first attempt at systematic characterization of psoriasis severity and aortic vascular inflammation. Our work provides strong evidence that psoriasis severity is linked with increased vascular inflammation beyond risk factors for vascular disease and, furthermore, that psoriasis severity predicts vascular inflammation beyond the Framingham risk score. These data suggest that psoriasis plaques are biologically active in promoting inflammation at remote sites. Our findings mirror epidemiological findings with respect to the relationship of cardiovascular and metabolic diseases with psoriasis severity. This study underscores the need to further our understanding of how skin severity relates to vascular inflammation and how this relationship changes with biological therapy.
Severity of Psoriasis Associates With Aortic Vascular Inflammation Detected by FDG PET/CT and Neutrophil Activation in a Prospective Observational Study
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MATERIALS AND METHODS

Study population. Participants were recruited from January 23, 2013 to March 24, 2015. The study consisted of a consecutive sample of 80 participants: sixty psoriasis participants and twenty age and gender matched healthy controls. Eligible psoriasis subjects had a diagnosis of psoriasis confirmed and quantified by a dermatologist using the Psoriasis Area Severity Index (PASI). Age and sex matched controls age 18-70 were consecutively recruited to undergo the same testing as the psoriasis subjects. Framingham risk scores were calculated. Financial compensation was provided to study participants. Study approval was granted by the National Heart, Lung and Blood Institute institutional review board in keeping with the Declaration of Helsinki. All study participants submitted written informed consent.

Psoriasis study inclusion/exclusion criteria. Inclusion criteria: Any patient with plaque psoriasis, aged 18-70 was included in the study. Exclusion criteria included disease states known to induce systemic and vascular inflammation, such as known cardiovascular disease, uncontrolled hypertension (systolic blood pressure > 180 mm Hg and diastolic blood pressure > 95 mm Hg), non-dermatological malignancy within 5 years, known HIV infection, active infection of any kind within the past 72 hours, major surgery within 3 months and history of intravenous drug use.

Healthy volunteer study criteria. Using IRB-approved materials including both paper and electronic advertisements, healthy volunteers were screened for exclusion criteria listed below. Participants were paid at the NIH Clinical Center rate for PET CT and blood draw. Control subjects were prospectively recruited as part of the healthy volunteer arm of an ongoing protocol studying inflammatory characterization of diabetes, coronary disease and healthy volunteers (NCT01934660) beginning August 28th, 2013. Our strategy was to recruit these controls matching by age (within five years) and gender to our psoriasis patients in a prospective fashion in blocks of five patients. For example, when 20 psoriasis patients were recruited, we summarized age and gender and then targeted recruitment of five controls to match those characteristics. At the time that we finished recruiting our sixty psoriasis subjects for this study (March 24th, 2015), we had recruited 22 healthy volunteers into our study. Because we decided a priori to use a 3:1 ratio between psoriasis and controls, we randomly excluded two subjects (both were 26 year old females) (Figure 1). For eligibility, we excluded individuals who were pregnant, breastfeeding, or had solid organ or hematologic malignancy (excluding nonmelanoma skin cancer), active infection within 3 months requiring antibiotics, collagen vascular diseases, clinical diagnosis of diabetes or cardiovascular disease, liver function tests greater than three times the upper limit of normal, estimated glomerular filtration rate<60 ml/min or body mass index >40 kg/m². Diabetes was defined as fasting glucose of ≥ 6.99 mmol/l, hemoglobin A1C > 6.5%, or use of diabetic medication. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg or use of anti-hypertensive medication. Hyperlipidemia was defined as total cholesterol >5.18 mmol/l, low-density lipoprotein (LDL) ≥ 4.14 mmol/l, or high-density lipoprotein (HDL) ≤ 1.04 mmol/l.

Clinical assessments. Demographic data including age, sex, relevant medical history including alcohol and tobacco status, history of hypertension, diabetes and hyperlipidemia were collected from all study participants. Characteristics of psoriasis severity including psoriasis duration and psoriasis therapy were collected from psoriasis participants. All psoriasis and controls underwent clinical, laboratory and imaging evaluation. Clinical parameters including blood pressure, height, weight, waist and hip circumferences were measured. Laboratory parameters including fasting blood glucose, fasting lipid panel, white blood count with differential, and systemic inflammatory markers including hsCRP and erythrocyte sedimentation rate (ESR) were evaluated in a clinical laboratory. Psoriasis severity was assessed by a dermatologist using PASI scoring. Homeostatic model assessment (HOMA) was used to quantify insulin resistance (IR) (HOMA-IR= (glucose (nmol/ml) + insulin (µIU/ml))/22.5).

Analysis of 18-FDG PET/CT and measuring vascular inflammation1,2. Following overnight fast PET/CT images were acquired approximately 60 minutes (mean 62 minutes ± 1) after administration of 10mCi 18-FDG. PET imaging occurred using a Siemens Biograph mCT PET/CT 64-slice scanner (Siemens Medical Solutions USA, Malvern, PA, USA), acquiring 1.5 mm axial slices of the aorta. Standard bed positions of three minutes each, scanning cranially to caudally were obtained for each patient from...
the vertex of the skull to the toes. Next, the 18-FDG uptake within the aorta was directly measured by utilizing dedicated analysis software for PET/CT (Extended Brilliance™ Workspace, Phillips Healthcare, Andover, MA, USA). Each region of interest produced two measures of metabolic activity: a mean standardized uptake value (SUV$_{\text{mean}}$) and maximal standardized uptake value (SUV$_{\text{max}}$), and these were obtained in the entire aorta from the aortic outflow tract to the abdominal aorta. Then, regions of interest were placed on 10 contiguous slices over the superior vena cava to obtain background activity of the FDG tracer. The SUV$_{\text{mean}}$ from each of the superior vena cava slices were then averaged to produce one venous value. To account for background blood pool activity, SUV$_{\text{max}}$ values from each aortic slice were divided by the venous SUV$_{\text{mean}}$, yielding a target-to-background ratio (TBR) (Supplemental Figure I).

**Plasma cytokine profiling.** Plasma levels of proteins known to associate with cardiovascular disease and neutrophil function were measured in EDTA-plasma collected from psoriasis or controls via a Meso Scale Discovery assay (MSD, Gaithersburg, MD).

**Neutrophil profiling.** In a subset of psoriasis patients who agreed to additional blood draws (n=42), venous blood was collected in sodium heparin vacutainers (Becton Dickinson (BD), San Jose, CA) for immunophenotyping by flow cytometry (Supplemental Table I). Erythrocytes were lysed using ACK lysing buffer (Quality Biologicals, Gaithersburg, MD) and cells were washed twice with FACS buffer (1X PBS, 0.5% bovine serum albumin, 0.02% EDTA). Cells were incubated with fluorochrome-conjugated antibodies for 30 minutes followed by a 10-minute incubation with LIVE/DEAD Aqua fixable viability dye (Life Technologies, Carlsbad, CA) (Supplemental Table I). Cells were washed with FACS buffer, fixed in 1% paraformaldehyde, and acquired on a BD Biosciences LSRII flow cytometer using DIVA 6.1.2 software (BD, San Jose, CA). Data was analyzed with FlowJo software version 9.7.6 (Treestar, La Jolla, CA) and results are presented as percent of viable cells. Serum levels of MPO (1:10 dilution, Abcam, USA), S100A8/A9 (MRP8/14) (1:20 dilution, Acris, USA), and NE-1 (1:100 dilution, Abcam, USA) were measured using enzyme linked immunostaining assays.

**Statistical analyses.** Descriptive statistics are presented as mean and standard deviation or median and interquartile range for continuous variables, and frequencies for categorical variables. Normality of distribution for continuous variables was assessed by skew and kurtosis and the dataset was deemed appropriate for parametric analysis. A Student’s t-test was used to compare parametric data (S100A8/A9) from psoriasis patients and controls. A Mann-Whitney U test was used to compare non-parametric data from psoriasis and controls. Relationships between PASI score, cardiometabolic risk factors and TBR were determined using Pearson’s correlation analysis and are reported as Pearson ρ (r) values. Unadjusted regression analysis was performed using TBR as the dependent variable and PASI score and cardiometabolic risk factors (age, male sex, current tobacco use, BMI, hypertension, hyperlipidemia, systolic blood pressure, HDL cholesterol, HOMA-IR and FRS) as independent variables, and standardized β-coefficients (β) and p values were reported for aortic TBR. Normality of distribution for neutrophil proteins assayed by ELISA was assessed by skew and kurtosis. A Student’s t-test was used to compare parametric data (S100A8/A9) from psoriasis patients and controls. A Mann-Whitney U test was used to compare non-parametric data from psoriasis and controls. Multivariate linear regression analysis was performed between PASI score and TBR, adjusting for demographic risk factors.
including age and sex (model 1); and established cardiovascular risk factors in the form of the Framingham risk score (model 2). We added each biomarker stepwise into the models with PASI score as outcome to understand how they relate to psoriasis severity and then to TBR as outcome to understand how they relate to vascular inflammation. All statistical analyses were performed using STATA 12.0 software (STATA Corp, College Station, TX, USA). A p value ≤0.05 was considered statistically significant for our primary analysis evaluating the relationship between TBR and PASI. Bonferroni correction for multiple testing was used for exploratory secondary analyses looking at whether three neutrophil biomarkers were associated with PASI and TBR. For these secondary analyses, p<0.0167 was considered statistically significant.

Sample size. We hypothesized that 5 point change in psoriasis severity measured by PASI score would lead to an increase in TBR of 0.1 with standard deviation equal to 0.1. Therefore, we required a group of 50 patients to have 90% power to test the relationship of psoriasis severity with aortic vascular inflammation. We followed the STROBE statement guidelines for reporting observational studies.4
REFERENCES


**Supplemental Table I:** Antibodies used for flow cytometry experiments.

<table>
<thead>
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<th>Antigen</th>
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<th>Clone</th>
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<td>OKT3</td>
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**Supplemental Table II**: Univariable regression analysis of vascular inflammation with cardiometabolic variables.

<table>
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<tr>
<td>Male sex</td>
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<tr>
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* β = Standardized regression coefficient; p ≤ 0.05 considered significant.
HOMA IR, homeostasis model assessment- insulin resistance; HDL, high density lipoprotein; PASI, psoriasis area and severity index.
Supplemental Figure I: FDG PET/CT analysis for quantification of vascular inflammation using EBW (Extended Brilliance Workstation, Philips Healthcare, USA). (A) shows a schematic diagram of the derivation of average Target-to-background ratio (TBR), the outcome measure of our study. 1) Ascending aorta, 2) Aortic arch, 3) Descending aorta, 4) Supra-renal abdominal aorta and 5) Infra-renal abdominal aorta. (B) shows a trans-axial 1.5 mm fused PET/CT slice at the level of the superior vena cava (SVC). Regions of interest (ROI) are drawn around the ascending and descending aorta as well as the SVC. SUVmean and SUVmax values obtained are used in the calculation of TBR as shown by (C).
Supplemental Figure II. Neutrophil characterization by flow cytometry. (A) After exclusion of debris, doublets, non-viable cells, and T and B cells markers, neutrophils were identified as CD3-CD19- CD15+SSC$^{\text{HI}}$. Positive gating was established using fluorescence minus one controls. Cell populations are presented as percentages of the parent population. (B) Neutrophil gating confirmed by backgating analysis.