

Endothelial Cell-, Platelet-, and Monocyte/Macrophage-Derived Microparticles are Elevated in Psoriasis Beyond Cardiometabolic Risk Factors

Junko Takeshita, MD, PhD; Emile R. Mohler, III, MD; Parasuram Krishnamoorthy, MD; Jonni Moore, PhD; Wade T. Rogers, PhD; Lifeng Zhang, PhD; Joel M. Gelfand, MD, MSCE; Nehal N. Mehta, MD, MSCE

Background—Psoriasis, especially when severe, is a risk factor for cardiometabolic disease beyond traditional risk factors. The mechanism of atherogenesis in psoriasis remains unknown. Cell membrane vesicles (ie, microparticles), released upon cell activation or apoptosis, have recently been associated with cardiometabolic disease and may play a pathogenic role. Microparticle levels, particularly from endothelial cells and platelets, are elevated in patients with cardiovascular disorders, metabolic syndrome, other inflammatory diseases, autoimmune conditions, and have been shown to be predictive of cardiovascular outcomes.

Methods and Results—Concentrations of microparticles with positive expression for any of 7 cell surface markers (Annexin V, CD3, CD31, CD41a, CD64, CD105, and CD144) were measured in blood samples from psoriasis patients (n=53) and control subjects without psoriasis (n=41). Platelet-free plasma was separated from whole blood by one-step centrifugation for microparticle analysis. Microparticles were fluorescently labeled and characterized by flow cytometry. Higher concentrations of CD105 (5.5/ μ L versus 2.5/ μ L, $P<0.001$), CD31 (31/ μ L versus 18/ μ L, $P=0.002$), CD41a (50/ μ L versus 22/ μ L, $P<0.001$), and CD64 (5.0/ μ L versus 4.1/ μ L, $P=0.02$) singly positive microparticles corresponding to endothelial cell-, platelet-, and monocyte/macrophage-derived microparticles, respectively, were found in psoriasis patients compared with controls. These differences persisted after adjustment for traditional cardiometabolic risk factors including body mass index.

Conclusions—Increased microparticle concentrations, independent of cardiometabolic risk factors, in patients with psoriasis suggest that the presence of increased endothelial cell, platelet, and monocyte/macrophage activation with cell turnover may contribute to the heightened atherogenesis associated with psoriasis. (*J Am Heart Assoc.* 2014;3:e000507 doi: 10.1161/JAHA.113.000507)

Key Words: atherosclerosis • inflammation • microparticles endothelium • platelets • psoriasis • risk factor

Microparticles (MPs) are membrane vesicles of 0.1 to 1 μ m in diameter generated from budding of the cell membrane. They contain nucleic acids, proteins, and other antigens from their cells of origin. These extracellular

vesicles are not inactive byproducts of damaged cell membranes but rather contributors to vascular pathology including inflammation, thrombosis, vascular reactivity, and angiogenesis.^{1,2} Leukocyte-derived MPs contain bioactive proteins such as interleukin 1, CD40 ligand, and intercellular adhesion molecule 1 (ICAM 1), which activate endothelial cells.^{3,4} Inflammation is also facilitated when MPs deliver arachidonic acid from activated circulating cells to the endothelium, which, in turn, leads to the adhesion and diapedesis of monocytes.⁵ Several types of MPs including leukocyte-derived MPs are present in atherosclerotic plaque.^{6,7} Leukocyte-derived MPs are part of the pathological process of unstable atherosclerotic plaques and are thought to contribute to plaque rupture and subsequent myocardial infarction or stroke.⁸ In addition to atherosclerosis,⁹ MPs are elevated in other chronic inflammatory conditions such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).^{10,11}

Psoriasis is a chronic inflammatory disease of the skin, which is associated with several cardiometabolic diseases

From the Section of Vascular Medicine (E.R.M.), Division of Cardiovascular Disease, Departments of Medicine (E.R.M., L.Z., N.N.M.), Dermatology (J.T., J.M.G.), and Pathology and Laboratory Medicine (J.M., W.T.R.), Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; Englewood Hospital and Medical Center, Englewood, NJ (P.K.); Section of Inflammation and Cardiometabolic Diseases, National Heart Lung and Blood Institute, Bethesda, MD (N.N.M.).

Correspondence to: Nehal N. Mehta, MD, MSCE, Section of Inflammation and Cardiometabolic Diseases, National Heart Lung and Blood Institute, Building 10, Bethesda 5-5140, MD. E-mail: nehal.mehta@nih.gov
Received August 28, 2013; accepted January 21, 2014.

© 2014 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley Blackwell. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

including obesity, dyslipidemia, diabetes, vascular inflammation, and major adverse cardiovascular events.^{12–19} Indeed, patients with more severe psoriasis have a shortened lifespan compared to those without psoriasis, and this is largely attributed to cardiovascular disease;²⁰ however, the underlying mechanisms of this link are poorly understood.

The inflammatory hypothesis of atherogenesis suggests that chronic inflammation accelerates atherosclerosis through repetitive vascular injury, and MPs may be involved in this process. Given that chronic inflammatory diseases such as SLE and RA are associated with increased production of MPs, it is likely that MPs are also elevated in patients with psoriasis considering that psoriasis is associated with increased immune activation, high epidermal cell turnover, and increased angiogenesis.²¹

Two small studies have observed an increase in endothelial cell- and platelet-derived MPs in patients with psoriasis.^{22,23} These studies were important initial contributions to understanding MPs in psoriasis; however, they were methodologically limited in MP detection by using ELISA-based assays²² and did not adjust for confounding variables known to modulate MPs such as cardiometabolic risk factors. Therefore, the aim of our study was to use a more rigorous method of MP detection using high-dimensional flow cytometry to measure and characterize circulating MPs in a well-phenotyped group of patients, thus enabling adjustment for cardiometabolic risk factors. We hypothesized that circulating plasma MPs are elevated in patients with psoriasis even after adjustment for cardiometabolic factors, and that the surface markers of these MPs are consistent with cells involved in atherogenesis, eg, monocytes/macrophages, endothelial cells, and platelets.

Methods

Study Design and Participant Protection

A cross-sectional study was performed to compare microparticle levels between patients with and without psoriasis. The study was approved by the University of Pennsylvania institutional review board, and informed consent was obtained from all subjects.

Study Population

Forty-one healthy control subjects and 53 patients with psoriasis were included in the study. Healthy subjects were recruited from the Philadelphia area and were excluded if they were active smokers or had hypertension, hyperlipidemia, or diabetes. Psoriasis patients were recruited between September 2011 and January 2012. Diagnosis of psoriasis was confirmed clinically by a dermatologist. Psoriasis severity was

measured by percent of body surface area (BSA) involved. Age, sex, height, weight, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), blood pressure, heart rate, and smoking status (never, past, current) were assessed on the date of recruitment for all subjects. Body mass index (BMI) was calculated using height and weight measurements. Psoriasis patients were further assessed for presence of diabetes, history of hypertension, use of anti-hypertensive medication, use of lipid-lowering medication, presence of psoriatic arthritis, and psoriasis treatment (topical, ultraviolet light, oral systemic, and/or biologic treatment).

Measurement of Microparticles

All samples were obtained, prepared, and stored as follows: 5 mL of venous blood were withdrawn using a 21G needle and collected in citrated tubes after discarding the first 1 mL of blood. Within 2 hours of collection, blood was centrifuged at 2500g for 15 minutes at room temperature to prepare platelet-poor plasma (PPP). The PPP was then collected, aliquoted, and stored at -80°C .

Reagents

FITC-Annexin V (Catalog No. 556570), PE-CD144 (Catalog No. 560410, clone 55-7H1), PerCP-Cy5.5-CD64 (Catalog No. 561194, clone 10.1), AF647-CD105 (Catalog No. 561439, clone 266), APC-H7-CD41a (Catalog No. 561422, clone HIP8), and V450-CD3 (Catalog No. 560365, clone UCHT1) were purchased from Becton Dickinson. PE-Cy7-CD31 (Catalog No. 303118, clone WM59) was purchased from Biolegend. Calibrator beads, 0.3 μm (Catalog No. LC-3) were purchased from Sigma. Calibrator beads, 1 μm (Catalog No. BCP-10-5) and 3 μm (Catalog No. BCP-30-5) were purchased from Spherotech. The antibodies were double-filtered before labeling with a 0.1 μm low protein-binding filter (Millipore, Cat# SLVV033RS). Aliquots of 25 μL of each sample were stained, after which 2.5 μL of 3.0 μm beads (equivalent to 25000 beads) was added to each tube as reference counting beads. Annexin Buffer (10 mmol/L HEPES, pH 7.4, 140 mmol/L NaCl, and 2.5 mmol/L CaCl_2) was added to each tube to make the total volume 250 μL . The Annexin Buffer was double-filtered by a 0.22 μm filter followed by a 0.1 μm filter.

Flow Cytometry

Samples were analyzed on a Special Order Research Product BD BioSciences FACS Canto A. The cytometer was calibrated daily with Cytometer Setup and Tracking (CS&T) Beads (BD) using Diva Software version 6.1.2. Forward and side scatter threshold, photomultiplier tube voltage, and window extension

were optimized to detect sub-micron particles. For each day that samples were analyzed, 1 tube containing only 0.3, 1, and 3- μm polystyrene size calibration beads was run at a fixed concentration. Area, height, and width forward scatter (FSC) and side scatter (SSC) parameters were analyzed and side scatter width (SSC-W) was found to best resolve the beads. The following instrument settings were used for data acquisition: threshold SSC 200; window extension 0.2; FSC voltage 200 V, SSC voltage 350 V; FSC and SSC in log scale. The acquisition was stopped when a fixed number of 3.0 μm beads (usually 20 000) were counted. Compensation tubes were also run using PPP, BD CompBead (BD Bioscience Cat# 552843), and were stained using the same reagents as were used in the sample tubes. For each sample, data were acquired simultaneously for all 7 reagents listed above (Annexin V, CD3, CD31, CD41a, CD64, CD105, and CD144) enabling the determination of any combination of marker expression for each individual microparticle. Moreover, a recorded event was considered to be a microparticle if and only if the following applied: (1) its SSC-W signal was less than that of the 1.0 μm size reference particles; and (2) it positively expressed at least 1 of the 7 fluorescent markers in the staining panel.

Microparticle number per μL of blood was calculated according to the following formula:

$$C_p = \frac{N_p}{N_b} \times \frac{25000 \text{ beads}}{25 \mu\text{L plasma}}$$

where C_p is the microparticle concentration (particles per μL plasma), N_p is the number of microparticles enumerated in a subset, and N_b is the number of reference counting beads counted in the sample. Samples were randomized into 2 batches, and each batch was prepared and analyzed within a single session on the flow cytometer.

Flow Cytometry Data Analysis

Analysis of the flow cytometry data was carried out using custom analysis programs written in the R Statistical Programming Environment²⁴ and using the Bioconductor open source software package flowCore.²⁵ For each of the 2 batches of samples, size calibration samples were run and SSC-W thresholds corresponding to the 1.0 μm beads were computed and applied to all samples within the batch. To determine positive expression of each of the 7 markers in the panel, all samples in a batch were aggregated, and the probability distributions of each of the 7 markers for the aggregated samples were examined. The Annexin V, CD31, and CD41a markers were bimodally distributed, indicating a clear distinction between positive and negative events. The other markers (CD3, CD64, CD105, and CD144) were unimodally distributed. For Annexin V, a kernel density

estimate (KDE) of the probability density was computed, and the minimum between modes was found. For CD31 and CD41a, the Annexin V negative subset of events was used to compute the KDE and the minimum between modes was found. For the remaining markers, the KDE of the Annexin V negative distribution was computed, and the location where the KDE fell to 0.01 of its maximum was used as a fiducial point. From that point a small constant offset in biexponential coordinates was added to arrive at the threshold for positive expression. Each of these “zero-point” thresholds was adjusted for spillover due to the intensities of all other markers by adding a value proportional to the square root of the spillover coefficient multiplied by the observed intensity of each marker. Figure shows the positivity thresholds resulting from this procedure for one of the batches (the other batch was qualitatively similar).

Characterization and quantification of the subcategories of MPs was performed using antibodies for select cell surface antigens to identify the cellular sources of the MPs. CD3 positivity was considered to be representative of T lymphocytes. CD31 positivity was used to identify a mixture of endothelial cells, platelets, and leukocytes. CD41a positivity was used as a more specific marker of platelets, and CD64 positivity was considered a more specific marker of monocytes/macrophages. CD144 positivity was used to identify a pure population of endothelial cells, and CD105 positivity identified activated endothelial cells. Annexin V positivity was identified regardless of the presence of other cell surface markers. Data presented for the remaining markers (CD3, CD31, CD41a, CD64, CD105, and CD144) represent MPs that are singly positive for each of these 6 markers and either Annexin V positive or negative. Therefore, CD31 positivity implies CD41a negativity and, in our presented data, identifies MPs of endothelial cell origin.

Statistical Analysis

Continuous data, with the exception of MP data, are presented as mean $\pm$ standard deviation if normally distributed and as median with interquartile range (IQR) if not normally distributed. Normality of continuous variables was assessed using tests for skewness and kurtosis. Means between 2 groups were compared using the 2-tailed, unpaired Student's *t* test. Medians between 2 groups were compared using the Wilcoxon rank-sum test. Qualitative data are presented as number of subjects with percentages. Frequencies between or among groups were compared using Fisher's exact test.

Multivariable linear regression of log-transformed MP concentrations was used to assess the association between each MP concentration and psoriasis status. Any MP measurements of zero were replaced with 0.5 before log-transformation. All models were adjusted for age, sex, BMI,

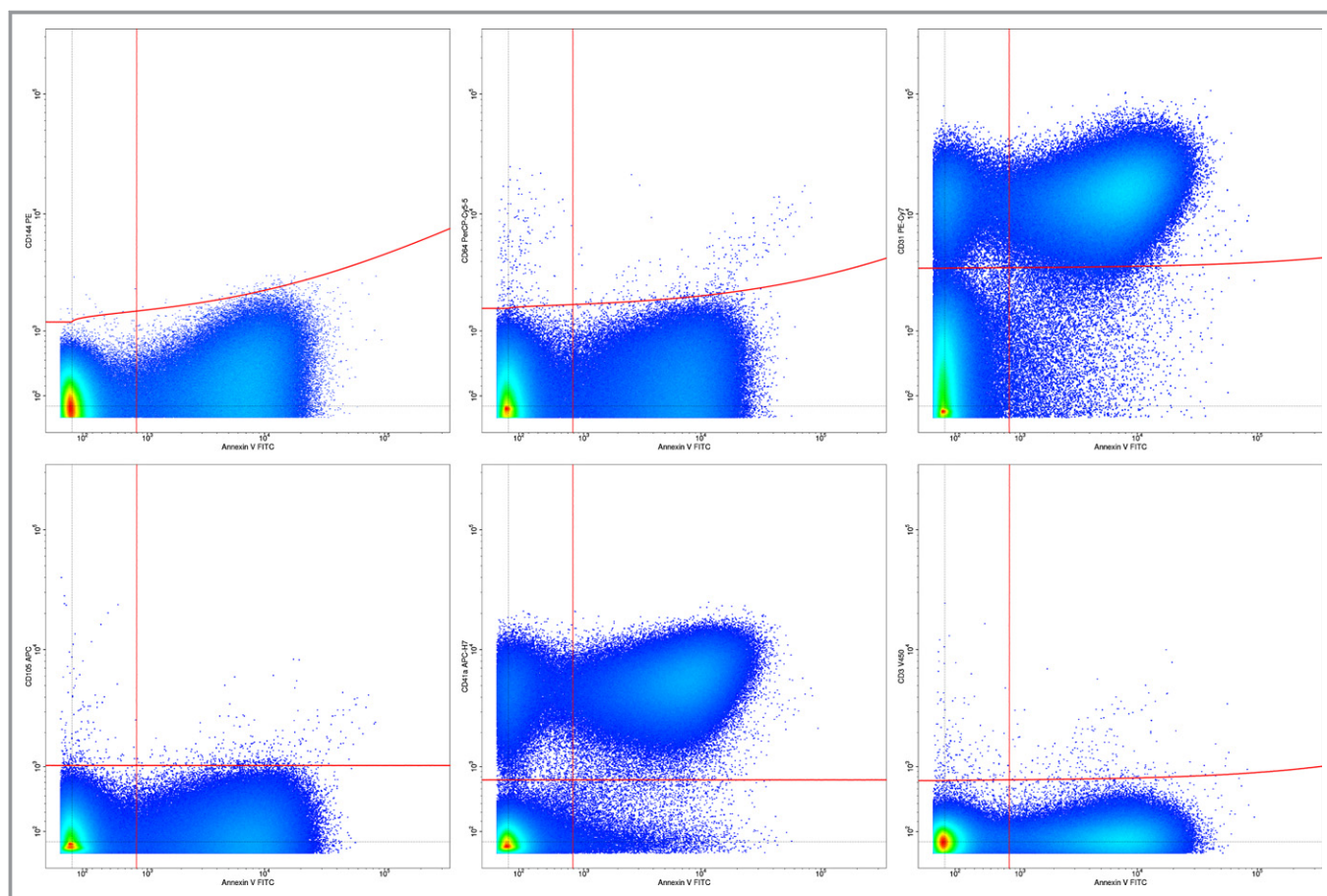


Figure. Fluorescence gating strategy. Microparticles were first gated on the Side Scatter Width signal at values less than or equal to the Side Scatter Width signal of 1.0 μm size reference beads. Positivity for each of the 7 markers in the panel was determined so as to take into account the spread of fluorescence due to spectral spillover. “Zero-point” thresholds (as described in the Methods section) were first determined and then variable adjustments proportional to the square root of the spillover signals were added to arrive at spill-adjusted thresholds (depicted as red curves) above or to the right of which an event was considered to positively express the indicated marker(s). The bivariate distributions shown are the aggregated data from the second of the 2 acquisition days (the first day was qualitatively similar). The x axis of each panel represents Annexin V fluorescein isothiocyanate (FITC). The y axis of each panel represents the remaining 6 markers.

systolic blood pressure (SBP), LDL, HDL, and triglyceride (TG) level, and smoking status (included in the model as former or never smokers since active smoking was an exclusion criterion for the healthy comparators) regardless of their statistical association with MP concentration and psoriasis status as these covariates have previously been shown to have significant associations with MP levels. Other measured potential confounders were not included in the multivariable model as they either were not significantly associated with MP concentration and psoriasis status (significance defined by $P < 0.1$) or did not change the β coefficient of the other parameters by more than 10%. Model diagnostics such as assessment of residual normality were performed to confirm adherence to assumptions of linear regression. MP concentration is presented as the predicted and adjusted mean and 95% confidence interval

(CI) as calculated from the multivariable linear regression model. The Benjamini-Hochberg procedure was used to adjust for multiple comparisons of 7 outcomes with significance defined as $P < 0.05$ in 2-sided tests. Sensitivity analyses excluding psoriasis patients with hypertension and taking anti-hypertensive medication, with hyperlipidemia and taking lipid-lowering medication, with diabetes, and who were active smokers were also performed.

Our study ($n=53$ versus $n=41$) provided $>90\%$ power to detect a difference in CD31+ microparticles between psoriasis and healthy subjects based on our hypothesis that we would observe differences similar to those in the published literature^{22,23} after adjusting for cardiometabolic risk factors. Except when stated otherwise, $P < 0.05$ was considered statistically significant. Statistical analysis was performed using STATA version 12.0 (StataCorp).

Results

Subject Characteristics

Fifty-three patients with psoriasis and 41 control subjects without psoriasis were included in the study. The psoriasis group was older (median age 59 versus 49, $P<0.001$), consisted of a higher proportion of males (60% versus 37%, $P=0.02$), had higher BMI (median BMI 30 versus 24, $P<0.001$), and had higher SBP (median SBP 131 versus 123, $P=0.006$) than healthy subjects. All lipid measurements (total cholesterol, LDL, HDL, and TG) were also significantly higher in the psoriasis group compared with the control group (Table 1). Among psoriasis patients, prevalence of cardiometabolic risk factors was as follows: 26% with hypertension, 34% with hyperlipidemia, and 11% with diabetes. Clinical characteristics of the psoriasis patients included 54.7% with mild psoriasis (ie, BSA $<3\%$), 28.3% with psoriatic arthritis, and 56.6% receiving oral systemic and/or biologic medication for treatment. Subject characteristics are detailed in Tables 1 and 2.

Table 1. Characteristics of Study Population

Variables	Psoriasis (N=53) Median (IQR)	Controls (N=41) Median (IQR)	P Value*
Male, N (%)	32 (60.4)	15 (36.6)	0.037 [†]
Age, y	49 (35, 62)	59 (55, 67)	<0.001
BMI	29.7 (25.0, 33.8)	23.8 (23.0, 26.2)	<0.001
SBP [‡] , mm Hg	131 (120, 141)	123 (112, 130)	0.006
DBP [‡] , mm Hg	76 (72, 86)	78 (72, 86)	0.67
Total cholesterol [‡]	189 (155, 216)	211 (186, 226)	0.025
LDL [‡]	107 (76, 128)	127 (117, 147)	<0.001
HDL [‡]	46 (41, 57)	59 (49, 75)	0.003
TG [‡]	166 (99, 204)	76 (45, 99)	<0.001
Smoking status [‡] , N (%)			
Never	31 (59.6)	27 (65.9)	0.82 ^{†,§}
Past	14 (26.9)	14 (34.1)	
Current	7 (13.5)	0 (0)	
Hypertension, N (%)	14 (26.4)	0 (0)	N/A
Diabetes, N (%)	6 (11.3)	0 (0)	N/A
Anti-hypertensive medication, N (%)	14 (26.4)	0 (0)	N/A
Lipid-lowering medication, N (%)	18 (34.0)	0 (0)	N/A

BMI indicates body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglyceride.

*Wilcoxon rank-sum test.

[†]Fisher's exact test.

[‡]Data for BMI, SBP, DBP, total cholesterol, LDL, HDL, TG, and smoking status were available for 49 to 52 (92.4% to 98.1%) subjects with psoriasis.

[§]Test of "Never" and "Past" smokers only.

Table 2. Psoriasis Characteristics

Characteristics	Psoriasis (N=53) Frequency (%)
Psoriasis extent by body surface area	
$<3\%$, mild	29 (54.7)
3% to 10%, moderate	21 (39.6)
$>10\%$, severe	3 (5.7)
Psoriatic arthritis	15 (28.3)
Psoriasis treatment*	
Topical only	15 (28.3)
Phototherapy	8 (16.7)
Oral systemic or biologic	30 (56.6)

*Percentages do not total 100% because some patients may be receiving more than one treatment.

Microparticle Levels in Patients With Psoriasis and Control Subjects Without Psoriasis

Of the 7 cell surface markers measured, patients with psoriasis were found to have significantly higher concentrations of CD105, CD31, CD41a, and CD64 singly positive MPs than healthy subjects in both unadjusted (data not shown) and adjusted analyses (Table 3). Among these MPs, concentrations were greatest for CD31 (31 MPs/ μ L; 95% CI, 24 to 39) and CD41a (50 MPs/ μ L; 95% CI, 39 to 63) positive MPs.

Table 3. Adjusted* Analysis of Microparticles

Microparticles [†]	Psoriasis (n=42) [‡] Mean (95% CI) count/ μ L	Control (n=41) Mean (95% CI) count/ μ L	P Value [§]
Annexin V+	5994 (4479 to 8022)	5208 (3948 to 6869)	0.58
CD3+	4.7 (3.6 to 6.1)	5.0 (3.9 to 6.4)	0.72
CD31+	31 (24 to 39)	18 (14 to 22)	0.002
CD41a+	50 (39 to 63)	22 (17 to 28)	<0.001
CD64+	5.0 (4.4 to 5.5)	4.1 (3.6 to 4.5)	0.02
CD105+	5.5 (4.4 to 6.7)	2.5 (2.0 to 3.0)	<0.001
CD144+	0.31 (0.23 to 0.39)	0.23 (0.18 to 0.29)	0.15

*Adjusted for age, sex, body mass index (BMI), systolic blood pressure (SBP), low-density lipoprotein (LDL) level, high-density lipoprotein (HDL) level, triglyceride (TG) level, and smoking history.

[†]Characterization and quantification of the subcategories of microparticles (MPs) was performed using antibodies for select cell surface antigens to identify the cellular sources of the MPs. CD3 identifies T lymphocytes; CD31, endothelial cells, platelets, and leukocytes (single positivity as presented above identifies endothelial cells); CD41a, platelets; CD64, monocytes/macrophages; CD105, activated endothelial cells; and CD144, pure endothelial cell population.

[‡]MP data were complete for 42 (79.2%) of 53 psoriasis patients. Of these 42 patients, 3 (7.1%) psoriasis patients had missing LDL, HDL and TG data; 1 (2.4%) patient had missing LDL data; and 1 (2.4%) patient had missing smoking history.

[§]P values corrected for multiple comparisons using the Benjamini-Hochberg procedure.

Collectively, these data support the greater presence of endothelial cell-, platelet-, and monocyte/macrophage-derived MPs, respectively, in patients with psoriasis compared to control subjects, independent of age, sex, BMI, SBP, LDL, HDL, TG, and smoking status. Concentrations of Annexin V positive MPs were similar in psoriasis patients and control subjects. No significant associations between any of the MP concentrations and psoriasis disease severity as determined by BSA affected were identified (range of β coefficients -0.30 to 0.15 ; each P for trend >0.05).

Sensitivity Analyses

Statistical analyses were repeated with only the “healthy” psoriasis patients by excluding any patients who were active smokers, had hypertension or diabetes, and were receiving anti-hypertensive or lipid-lowering medications ($N=17$). Concentrations of CD105, CD31, and CD41a singly positive MPs in the “healthy” psoriasis patients remained significantly higher than those in the control subjects (Table 4). While CD64 singly positive MP concentrations also remained higher in “healthy” psoriasis patients than in control subjects, the difference was no longer statistically significant. MP concentrations were also compared between patients with psoriasis only and patients with psoriatic arthritis, and no significant differences in MP concentrations between the 2 groups were found (data not shown).

Table 4. Sensitivity Analysis^{*,†}

Microparticles [‡]	Psoriasis (n=17) [§] Mean (95% CI) count/ μ L	Control (n=41) Mean (95% CI) count/ μ L	P Value
Annexin V+	7803 (4954 to 12 290)	5208 (3993 to 6791)	0.18
CD3+	4.5 (3.0 to 6.8)	5.0 (3.9 to 6.3)	0.69
CD31+	34 (22 to 49)	18 (14 to 22)	0.01
CD41a+	70 (47 to 103)	22 (17 to 28)	<0.001
CD64+	4.9 (4.0 to 5.8)	4.1 (3.6 to 4.5)	0.15
CD105+	5.6 (4.0 to 7.8)	2.5 (2.0 to 3.0)	<0.001
CD144+	0.32 (0.21 to 0.47)	0.23 (0.18 to 0.29)	0.20

*Exclusion of psoriasis subjects with history of hypertension, diabetes, use of lipid-lowering medication, and current smoking.

†Adjusted for age, sex, body mass index (BMI), systolic blood pressure (SBP), low-density lipoprotein (LDL) level, high-density lipoprotein (HDL) level, triglyceride (TG) level, and smoking history.

‡Characterization and quantification of the subcategories of microparticles (MPs) was performed using antibodies for select cell surface antigens to identify the cellular sources of the MPs. CD3 identifies T lymphocytes; CD31, endothelial cells, platelets, and leukocytes (single positivity as presented above identifies endothelial cells); CD41a, platelets; CD64, monocytes/macrophages; CD105, activated endothelial cells; and CD144, pure endothelial cell population.

§Data were complete for 14 (82.4%) of the 17 “healthy” psoriasis patients.

||P values corrected for multiple comparisons using the Benjamini-Hochberg procedure.

Discussion

In this cross-sectional study, we demonstrate that endothelial cell-, platelet-, and, to a lesser extent, monocyte/macrophage-derived MPs as identified by CD105, CD31, CD41a, and CD64 singly positive MPs, respectively, are significantly elevated in patients with psoriasis compared with control subjects without psoriasis, independent of traditional cardiometabolic risk factors. Our study confirms previous reports of increased circulating endothelial cell- and platelet-derived MP levels in patients with psoriasis^{22,23} and adds to those findings by adjusting for the presence of cardiometabolic risk factors such as age, sex, blood pressure, lipid levels, and smoking status that were not completely accounted for in prior studies.

Human CD31+/platelet endothelial cell adhesion molecule (PECAM)-1 is a surface marker expressed by monocytes, polymorphonuclear cells, endothelial cells, platelets, and a discrete population of circulating lymphocytes, all of which are important cellular components of atherosclerosis.²⁶ The potential significance of CD31 expression in atherogenesis and cardiovascular disease is also suggested by CD31's function as an adhesion molecule receptor that facilitates leukocyte trafficking across the endothelial layer.²⁷ In fact, high CD31-positive MP levels have been reported to independently predict poor cardiovascular outcomes in patients with coronary artery disease.²⁸ We identify the presence of increased levels of the endothelial cell-derived CD31-positive MPs in patients with psoriasis and, together with results from prior studies, our findings suggest that CD31-positive MPs, particularly endothelial cell-derived subsets, may be highly relevant in further characterizing the association between psoriasis and cardiometabolic disease.

Platelet CD41/CD61 complex, also known as platelet glycoprotein (GP) IIb/IIIa, or integrin α IIb β 3, mediates platelet aggregation by serving as the receptor for fibrinogen and von Willebrand factor. In the setting of an acute coronary artery ischemic event, activated platelets produce CD41 positive platelet-derived MPs that bind to the endothelium, submatrix of the vascular wall, and leukocytes, and thereby facilitate thrombus propagation.^{29,30} The platelet-derived MPs can also inflict endothelial cell damage via induction of inflammation and impairment of endothelial-dependent vasodilation.³¹ Our finding of elevated CD41a MP concentrations among patients with psoriasis supports the concept that the psoriatic state increases risk of cardiometabolic events via detrimental effects of MPs on endothelial function and enhanced platelet-derived thrombosis.

Collectively, the results of our study show that patients with psoriasis have systemic evidence of increased cell turnover and activation particularly of endothelial cells,

platelets, and monocytes/macrophages, providing further evidence that the inflammatory effects of psoriasis are more than just skin deep. The exact nature of the significance of these findings, however, remains unclear. While there is ample *in vitro* evidence of the potential downstream biological effects of MPs (eg, promotion of coagulation, vascular dysfunction, regulation of inflammation),^{32,33} many of which are known to be important in atherogenesis, *in vivo* data are few in patients with psoriasis at this juncture.

There are several potential limitations of our study worth noting, namely its cross-sectional design and inability to determine directionality, single-center nature, and use of control subjects chosen from a different source population than the psoriasis patients. Furthermore, the study design did not allow for determination of an association between MP levels and psoriasis severity. A previous study did report a significant correlation between platelet-derived MP levels and psoriasis severity as measured by the Psoriasis Area and Severity Index (PASI),²² and we suspect the absence of an association in our study is at least partially attributable to the fact that the majority of our psoriasis participants either had inherently mild disease or had history of severe disease on effective therapy.

In conclusion, our findings support the concept that MPs may be pathogenic in psoriasis patients. However, future studies are needed to address whether MPs are simply biomarkers of inflammatory disease or have important *in vivo* biological activity that contributes to the development and progression of cardiometabolic disease in psoriasis.

Sources of Funding

This work was partly funded by a grant from the Intramural Research Program at the NIH (Mehta), Training Grant T32 GM 075766-6 (Takeshita), a National Psoriasis Foundation Fellowship Award (Takeshita), a Dermatology Foundation Career Development Award (Takeshita), a K24-AR064310 grant from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (Gelfand), and a R01-HL111293 grant from the National Heart Lung and Blood Institute.

Disclosures

Drs Mohler, Moore, and Rogers declare financial interest in CytoVas, LLC, a company engaged in developing cell-based biomarkers for vascular health. Dr Gelfand has served as a consultant for Abbvie, Amgen Inc, Eli Lilly, Celgene Corp, Merck, Janssen Biologics (formerly Centocor), Novartis Corp, and Pfizer Inc, receiving honoraria; had grants or has pending grants from Abbvie, Amgen Inc, Genentech Inc, Novartis Corp, Eli Lilly, and Pfizer Inc; and received payment for continuing medical education work related to psoriasis.

References

- Curtis AM, Edelberg J, Jonas R, Rogers WT, Moore JS, Syed W, Mohler ER. Endothelial microparticles: sophisticated vesicles modulating vascular function. *Vasc Med*. 2013;18:204–214.
- Sandberg H, Bode AP, Dombrose FA, Hoechli M, Lentz BR. Expression of coagulant activity in human platelets: release of membranous vesicles providing platelet factor 1 and platelet factor 3. *Thromb Res*. 1985;39:63–79.
- Wang JG, Williams JC, Davis BK, Jacobson K, Doerschuk CM, Ting JP, Mackman N. Monocytic microparticles activate endothelial cells in an IL-1 β -dependent manner. *Blood*. 2011;118:2366–2374.
- Leroyer AS, Rautou PE, Silvestre JS, Castier Y, Leseche G, Devue C, Duriez M, Brandes RP, Lutgens E, Tedgui A, Boulanger CM. CD40 ligand+ microparticles from human atherosclerotic plaques stimulate endothelial proliferation and angiogenesis: a potential mechanism for intraplaque neovascularization. *J Am Coll Cardiol*. 2008;52:1302–1311.
- Andriantsitohaina R, Gaceb A, Vergori L, Martinez MC. Microparticles as regulators of cardiovascular inflammation. *Trends Cardiovasc Med*. 2012;22:88–92.
- Mayr M, Grainger D, Mayr U, Leroyer AS, Leseche G, Sidibe A, Herbin O, Yin X, Gomes A, Madhu B, Griffiths JR, Xu Q, Tedgui A, Boulanger CM. Proteomics, metabolomics, and immunomics on microparticles derived from human atherosclerotic plaques. *Circ Cardiovasc Genet*. 2009;2:379–388.
- Rautou PE, Leroyer AS, Ramkhalawon B, Devue C, Dufaut D, Vion AC, Nalbone G, Castier Y, Leseche G, Lehoux S, Tedgui A, Boulanger CM. Microparticles from human atherosclerotic plaques promote endothelial ICAM-1-dependent monocyte adhesion and transendothelial migration. *Circ Res*. 2011;108:335–343.
- Sarlon-Bartoli G, Bennis Y, Lacroix R, Marti P, Bartoli MD, Arnaud L, Mancini J, Boudes A, Sarlon E, Thevenin B, Leroyer AS, Squarioni C, Magnan PE, Dignat-George F, Sabatier F. Plasmatic level of leukocyte-derived microparticles is associated with unstable plaque in asymptomatic patients with high-grade carotid stenosis. *J Am Coll Cardiol*. 2013;62:1436–1441.
- Kurtzman N, Zhang L, French B, Jonas R, Bantly A, Rogers WT, Moore JS, Rickels MR, Mohler ER. Personalized cytomic assessment of vascular health: evaluation of the vascular health profile in diabetes mellitus. *Cytometry B Clin Cytom*. 2013;84:255–266.
- Distler JH, Beyer C, Schett G, Luscher TF, Gay S, Distler O. Endothelial progenitor cells: novel players in the pathogenesis of rheumatic diseases. *Arthritis Rheum*. 2009;60:3168–3179.
- Beyer C, Pisetsky DS. The role of microparticles in the pathogenesis of rheumatic diseases. *Nat Rev Rheumatol*. 2010;6:21–29.
- Armstrong AW, Harskamp CT, Armstrong EJ. The association between psoriasis and obesity: a systematic review and meta-analysis of observational studies. *Nutr Diabetes*. 2012;2:e54. doi: 10.1038/nutd.2012.26
- Ma C, Harskamp CT, Armstrong EJ, Armstrong AW. The association between psoriasis and dyslipidaemia: a systematic review. *Br J Dermatol*. 2013;168:486–495.
- Armstrong AW, Harskamp CT, Armstrong EJ. Psoriasis and the risk of diabetes mellitus: a systematic review and meta-analysis. *JAMA Dermatol*. 2013;149:84–91.
- Langan SM, Seminara NM, Shin DB, Troxel AB, Kimmel SE, Mehta NN, Margolis DJ, Gelfand JM. *J Invest Dermatol*. 2012 Mar;132(3 Pt 1):556–562. doi: 10.1038/jid.2011.365. Epub 2011 Nov 24.
- Mehta NN, Yu Y, Saboury B, Foroughi N, Krishnamoorthy P, Raper A, Baer A, Antigua J, Van Voorhees AS, Torigan DA, Alavi A, Gelfand JM. Systemic and vascular inflammation in patients with moderate to severe psoriasis as measured by [18F]-fluorodeoxyglucose positron emission tomography-computed tomography (FDG-PET/CT): a pilot study. *Arch Dermatol*. 2011;147:1031–1039.
- Gelfand JM, Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB. Risk of myocardial infarction in patients with psoriasis. *JAMA*. 2006;296:1735–1741.
- Gelfand JM, Dommasch ED, Shin DB, Azfar RS, Kurd SK, Wang X, Troxel AB. The risk of stroke in patients with psoriasis. *J Invest Dermatol*. 2009;129:2411–2418.
- Mehta NN, Azfar RS, Shin DB, Neimann AL, Troxel AB, Gelfand JM. Patients with severe psoriasis are at increased risk of cardiovascular mortality: cohort study using the General Practice Research Database. *Eur Heart J*. 2010;31:1000–1006.
- Mehta NN, Pinnelas R, Krishnamoorthy P, Yu Y, Shin DB, Troxel AB, Gelfand JM. Attributable risk estimate of severe psoriasis on major cardiovascular events. *Am J Med*. 2011;124:775.e1–e6.
- Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med*. 2009;361:496–509.
- Tamagawa-Mineoka R, Katoh N, Kishimoto S. Platelet activation in patients with psoriasis: increased plasma levels of platelet-derived microparticles and soluble P-selectin. *J Am Acad Dermatol*. 2010;62:621–626.

23. Pelletier F, Garnache-Ottou F, Angelot F, Biichle S, Vidal C, Humbert P, Saas P, Seilles E, Aubin F. Increased levels of circulating endothelial-derived microparticles and small-size platelet-derived microparticles in psoriasis. *J Invest Dermatol*. 2011;131:1573–1576.
24. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*. 2004;5:R80.
25. Hahne F, LeMeur N, Brinkman RR, Ellis B, Haaland P, Sarkar D, Spidlen J, Strain E, Gentleman R. flowCore: a Bioconductor package for high throughput flow cytometry. *BMC Bioinformatics*. 2009;10:106.
26. DeLisser HM, Newman PJ, Albelda SM. Molecular and functional aspects of PECAM-1/CD31. *Immunol Today*. 1994;15:490–495.
27. Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. *J Exp Med*. 1993;178:449–460.
28. Sinning JM, Losch J, Walenta K, Bohm M, Nickenig G, Werner N. Circulating CD31+/Annexin V+ microparticles correlate with cardiovascular outcomes. *Eur Heart J*. 2011;32:2034–2041.
29. Merten M, Pakala R, Thiagarajan P, Benedict CR. Platelet microparticles promote platelet interaction with subendothelial matrix in a glycoprotein IIb/IIIa-dependent mechanism. *Circulation*. 1999;99:2577–2582.
30. Shantsila E, Kamphuisen PW, Lip GY. Circulating microparticles in cardiovascular disease: implications for atherogenesis and atherothrombosis. *J Thromb Haemost*. 2010;8:2358–2368.
31. Boulanger CM, Scoazec A, Ebrahimian T, Henry P, Mathieu E, Tedgui A, Mallat Z. Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. *Circulation*. 2001;104:2649–2652.
32. VanWijk MJ, VanBavel E, Sturk A, Nieuwland R. Microparticles in cardiovascular diseases. *Cardiovasc Res*. 2003;59:277–287.
33. Chironi GN, Boulanger CM, Simon A, Dignat-George F, Freyssinet JM, Tedgui A. Endothelial microparticles in diseases. *Cell Tissue Res*. 2009;335:143–151.

Endothelial Cell-, Platelet-, and Monocyte/Macrophage-Derived Microparticles are Elevated in Psoriasis Beyond Cardiometabolic Risk Factors

Junko Takeshita, Emile R. Mohler, Parasuram Krishnamoorthy, Jonni Moore, Wade T. Rogers, Lifeng Zhang, Joel M. Gelfand and Nehal N. Mehta

J Am Heart Assoc. 2014;3:e000507; originally published February 28, 2014;

doi: 10.1161/JAHA.113.000507

The *Journal of the American Heart Association* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Online ISSN: 2047-9980

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://jaha.ahajournals.org/content/3/1/e000507>