Original Paper

Determinants of Vascular Function in Patients With Chronic Gout

Robert D. Brook, MD;[1] Srilakshmi Yalavarthi, MS;[1] James D. Myles, PhD;[2] Shokoufeh Khalatbari, MS;[2] Rita Hench, CCRC;[1] Susan Lustig;[1,*] Wendy Marder, MD;[1] Adam Neidert, BS;[1] Mariana J. Kaplan, MD[1]

Epidemiologic studies have proposed a relationship between hyperuricemia and cardiovascular (CV) risk. However, it is unclear whether uric acid (UA) is an independent risk factor for CV disease (CVD) after controlling for other predisposing conditions. Gout patients might have persistent systemic inflammation, which, in addition to hyperuricemia, may potentiate CVD. This study examined vascular function and markers of CV damage in gout patients when compared with healthy controls. Brachial artery flow-mediated dilatation, arterial compliance, and microvascular function were measured. Circulating apoptotic endothelial cells and endothelial progenitor cells were quantified by FACS and circulating biomarkers of CVD by enzyme-linked immunosorbent assay. Gout patients displayed significant increases in body mass index, C-reactive protein, UA, and triglycerides and decreases in high-density lipoprotein. There were no significant differences in other CV traditional risk factors, adhesion molecules, or chemokines. Gout patients did not differ from controls in vascular function. In univariate and multivariate analysis, UA was not associated with the quantified CV risk parameters. Despite an increase in several CV risk factors, inflammation, and UA, gout patients display normal endothelial function and no increases in biomarkers of CVD. These results do not support the notion that gout is an independent risk factor for premature CVD.
J Clin Hypertens (Greenwich). *2011;13:178–188.*

From the Divisions of Rheumatology and Cardiology, Department of Internal Medicine;[1] and the Michigan Institute for Clinical Research, University of Michigan Medical School, Ann Arbor, MI[2]
**Research Vascular Laboratory, Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, MI*
Address for correspondence: Mariana J. Kaplan, MD, Division of Rheumatology, University of Michigan Medical School, 1150 W. Medical Center Dr., 5520 Medical Sciences Research Building I, Ann Arbor, MI 48109-5680
E-mail: makaplan@umich.edu
Manuscript received August 23, 2010; revised October 26, 2010; accepted October 28, 2010

doi: 10.1111/j.1751-7176.2010.00406.x

Gouty arthritis is a very common condition characterized by chronic hyperuricemia with periods of intense inflammation, secondary to monosodium urate crystal deposition in joints and soft tissues. Epidemiologic evidence suggests an association between elevated serum uric acid (UA) levels and increased cardiovascular (CV) morbidity and mortality.[1,2] A direct causal role, however, remains to be established. Given the association of hyperuricemia with various other comorbidities, it is unclear whether UA is an independent risk factor for cardiovascular disease (CVD). Some studies suggest that, while high UA levels may be an independent risk factor for CVD in high-risk individuals, the magnitude of this risk attributable to serum UA is likely to be small in healthy persons.[3,4] The role of hyperuricemia in cardiovascular (CV) complications also appears to differ between men and women.[5] Further, no biomarkers of CV risk in patients with conditions associated with chronic hyperuricemia, such as gout, have been established in a systematic manner.

In chronic gout, the combination of persistent systemic and joint inflammation and hyperuricemia may potentiate or synergize CVD development.[6] It has been proposed that urate crystal material in vessel walls may cause neutrophil and platelet activation and release of inflammatory cytokines, acute-phase reactants, chemokines, and adhesion molecules that are known to promote CV damage.[7–9] It is therefore possible that factors other than serum UA play a role in promoting CVD in chronic gout.

It remains unclear, however, whether gout confers an increased CV risk when controlling for traditional risk factors and whether this risk is higher than in individuals with asymptomatic hyperuricemia. The confusion with regards to UA's role in CVD promotion is also enhanced by the various potentially deleterious and homeostatic roles that this molecule has in vascular biology.

The endothelium plays a pivotal role in CV regulation through release of nitric oxide (NO), which causes vasodilatation, inhibits platelet aggregation, and reduces inflammation. Impaired blood flow responses to endothelium-dependent vasodilators, both at the conduit and smaller blood vessels, are characteristic in patients with various CV risk factors and are considered important early steps in atherosclerosis development.[10] Disruption of endothelium-dependent NO bioavailability manifests as reduced large artery compliance and impaired baroreflex sensitivity.[11] We hypothesize that, since chronic and/or recurrent acute gout is associated with systemic inflammation, it could lead to endothelial dysfunction and decreased arterial compliance, similar to what has been found in other chronic arthritides including rheumatoid arthritis and ankylosing spondylitis.[12,13]

This study examined conduit and microvascular endothelial and vascular smooth muscle (VSM) function in patients with gout but otherwise low Framingham risk scores, and analyzed whether markers of CV damage and repair are abnormal in patients with this condition when compared with healthy controls. We also analyzed whether various blood biomarkers of vascular damage were associated with endothelial dysfunction in patients with gout and otherwise low prevalence of CV risk factors.

MATERIAL AND METHODS

Patients

The University of Michigan institutional review board approved this cohort association study, which complied with the Declaration of Helsinki. Patients signed informed consent. Adult patients with a diagnosis of chronic gout (n=20) who met the 1977 American Rheumatism Association (ARA) criteria for gout[14] were recruited from the rheumatology out-patient clinic and by advertisement. Controls (n=20) were recruited to match for age and sex of gout patients. Any level of serum UA was permitted for both groups. Patients were excluded if they had other inflammatory arthritides or systemic autoimmune diseases, diabetes mellitus, were smokers or on statins, had active infections, were pregnant, or had evidence of significant liver or renal disease (creatinine clearance <65 mL/min/1.73 m^2). Patients with transplantation-induced gout on immunosuppressive medications and patients receiving angiotensin receptor blockers (ARBs) were excluded. To exclude the effects of drugs used to treat hyperuricemia and/or gout in endothelial function measurements, patients taking any uricosuric agent and/or xanthine oxidase (XO) inhibitor underwent drug washout for 1 month prior to measurements of vascular function and biomarkers. Patients taking corticosteroids, nonsteroidal anti-inflammatory drugs or colchicine underwent a washout period of 2 weeks before testing. Acetaminophen was allowed during the washout period if clinically necessary. If patients underwent drug washout, serum UA was rechecked on the day that endothelial function measurements were assessed.

Vascular Procedures

Procedures were performed in the Vascular Laboratory, University of Michigan, in a temperature-controlled room, using the arm contralateral to the one used for venipuncture. Patients were asked to fast for 12 hours prior to evaluation.

Pulse Wave Analyses and Arterial PWV to Assess Arterial Compliance

After resting supine for 10 minutes, dominant-arm radial artery applanation tonometry (AT) was sequentially measured at the right carotid and femoral arteries for 10 seconds each, following SphygmoCor guidelines (Atcor, Itasca, IL). Three-lead electrocardiography (ECG) recordings were simultaneously obtained with AT to calculate pulse wave velocity (PWV). Semi-automated results were provided by the device's internal calculations, including: (1) central aortic systolic, diastolic, and pulse pressures; (2) aortic augmentation pressure (AP), augmentation index (AIx), and AIx standardized to a heart rate of 75 beats per minute (AIx@75); (3) ejection duration and subendocardial viability ratio;

and (4) aortic PWV (a direct measure of arterial stiffness).

Concomitant Microvascular and Conduit Brachial Endothelial Function Protocol

Simultaneous measurement of conduit artery endothelial-dependent vasodilatation by brachial flow-mediated dilatation (FMD) using ultrasound and of microvascular endothelial-dependent vasodilatation by the semi-automated EndoPat-2000 device (Itamar, Caesarea, Israel [http://www.itamar-medical.com]) was performed on the dominant arm. After completion of SphygmoCor readings, patients rested in a supine position for 10 minutes and were then connected to the EndoPat-2000 device finger probes and to a 3-lead ECG system to perform ECG-triggered B-mode brachial artery measurements by ultrasound. Arterial B-mode ultrasonography of the ipsilateral dominant arm was performed concomitant to the EndoPAT-2000 protocol (see below).

Microvascular Endothelial Function

The EndoPAT-2000 uses finger pulse amplitude tonometry (PAT), which is measured before (baseline) and after reactive hyperemia (RH), termed RH-PAT, induced by upper-arm cuff occlusion for 5 minutes. Five minutes of bilateral basal resting finger PAT was recorded on one finger of each hand by EndoPat probes. At minute 4, basal brachial artery diameter (BAD) was recorded by Duplex ultrasound in the dominant arm for 10 seconds, to be used for brachial FMD measurement. A dominant upper-arm blood pressure (BP) cuff was inflated to 50 mm Hg above systolic BP to occlude blood flow for 5 minutes. Upon rapid cuff deflation, RH-PAT was recorded in the ipsilateral dominant hand finger for 5 minutes post-BP cuff release. The device's computer compared 120 seconds of baseline mean PAT (during the initial 5 minutes period on dominant arm) with the RH-PAT, defined as the mean PAT from 60 to 120 seconds post-cuff release on the same dominant arm. These readings were standardized to PAT of the contralateral hand during these periods to provide RH-PAT index (RI). Concomitantly, at 50 to 90 seconds post-release of BP cuff during reactive hyperemia period, BAD was continuously recorded by Duplex ultrasound to calculate brachial artery FMD (see below).

At the end of the second post-cuff deflation 5-minute period, patients were administered nitroglycerin (0.4 mg sublingual). Nondominant arm finger PAT was recorded for 6 minutes. The PAT level for a 1-minute period occurring between 5 and 6 minutes after nitroglycerin was recorded and compared with a 1-minute period in the same nondominant arm during the original baseline period prior to RH-PAT. This provided nitroglycerin-mediated PAT index (NTG-PAT) to assess endothelial-independent VSM-mediated dilatation. If systolic pressure was <100 mm Hg, the patient was orthostatic and/or had a history of reaction to nitroglycerin, nitroglycerin-mediated vasodilatation (NMD) procedure was not performed.

Brachial FMD by Duplex Ultrasound

The protocol is in accord with published guidelines and as reported by our group.[15,16] A modified portable computer Terason2000 ultrasound system (Burlington, MA) with a 10.0-mHz linear array transducer was used to simultaneously determine brachial vasoreactivity with measurement of RH-PAT index. Three ECG leads were placed on the patients' chest and attached to a portable DASH 300 ECG monitor (GE Healthcare, Waukesha, WI) connected to the ultrasound system and sent a standard voltage output for the ultrasound to trigger image acquisition upon the ECG R wave. This was done prior to vascular reactivity testing. BAD was longitudinally imaged by B-mode imaging with the transducer 2 to 10 cm above the antecubital crease on the dominant arm, and all subsequent images were obtained at arm location. All images were acquired at the end of each R wave on the ECG by a triggered event and directly stored on a computer in digital Diacom format. Approximately 10 seconds were acquired during the 4th minute of resting basal finger PAT measurement by EndoPAT. A BP cuff was inflated to 50 mm Hg above systolic BP over the proximal upper arm portion of the ipsilateral upper arm and rapidly deflated after 5 minutes of occlusion. Repeat longitudinal B-mode ultrasound measurements of the identical location of the brachial artery were continuously imaged from 50 to 90 seconds post-cuff release. FMD was defined as the percent change in BAD from baseline in response to reactive hyperemia (measured from media-adventia line [M line] to M line). FMD= [(reactive hyperemia BAD – basal BAD)/basal BAD]×100 (in %). Image analyses for BAD and FMD were performed using software from Medical Imaging Applications, Inc (Coralville, IA).

Measurement of Serum and Plasma Markers of Vascular Damage

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to quantify

serum or plasma levels of intracellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), plasminogen activator inhibitor-1 (PAI-1), tissue factor (TF), and interleukin (IL)-6 (all from R&D Systems, Minneapolis, MN); monocyte chemoattractant protein-1 (MCP-1) (BD-Bioscience, San Jose, CA); tissue plasminogen activator (TPA) (Innovative Research, Novi, MI), following manufacturer's instructions. A PAI-1/TPA ratio was calculated.

Quantification of Circulating Apoptotic Endothelial Cells and Circulating EPCs

This was performed as described by our group.[16,17] Peripheral blood was obtained with the first 2 mL of blood drawn discarded to avoid contamination by endothelial cells from the punctured vessel wall. Peripheral blood mononuclear cells (PBMCs) were isolated with Ficoll-Hypaque gradient, washed, resuspended in PBS, 2% horse serum and sodium azide, and incubated with fluorochrome-conjugated antibodies that identify cells as endothelial (anti-CD146-PE) and determinants of apoptosis (Annexin-APC) or their isotype controls (all from BD Biosciences), while excluding lineage-positive cells. To quantify circulating endothelial progenitor cells (EPCs), PBMCs were incubated with PE-CD133 (Miltenyi Biotech, Auburn, CA) and APC-CD34 in combination with a cocktail of PE/cy5-conjugated antibodies recognizing CD3, CD79b, and CD56 (BD Biosciences). Immunofluorescence was measured using a Coulter XL flow cytometer (Hialeah, FL). EPCs were identified in the lymphocyte population as $CD34^+/CD133^+$ cells in the $CD3^-/CD79b^-/CD56^-$ gate. The number of positive events detected by FACS was divided by the sum of lymphocytes plus monocyte events obtained during the acquisition. The percentage of $CD34^+/CD133^+$ cells in the PBMC pool (CD34/CD133 divided by lymphocytes plus monocytes) was used to calculate the total number of $CD34^+/CD133^+$ cells recovered from the PBMC isolation and then divided by the initial blood volume to determine $CD34^+/CD133^+$ cells/mL blood.

Other Laboratory Measurements

Lipids, C-reactive protein (CRP), fasting glucose, serum urea nitrogen (BUN), and creatinine were measured at the Central Laboratories of the University of Michigan using standardized techniques.

Statistical Analysis

Continuous variables were summarized using means and standard deviations or ranges and compared between the two cohorts using Student *t* test or Wilcoxon rank sum test if there was lack of normality. Categoric variables were summarized by counts and proportions and compared between the two cohorts using Chi-square or Fisher exact test. Pearson or Spearman Correlation Coefficients were used to examine correlation between the variables. Multivariate regression analyses were performed to identify independent predictors of vascular dysfunction. Using the Spearman correlation analysis and Wilcoxon rank sum test accounted for non-normality of variables. All inference results presented in Table I and Table II are based on the *t* test or Wilcoxon rank sum test. Framingham risk scores were calculated using the md+calc Web site (http://www.mdcalc.com/framingham-cardiac-risk-score).

We arrived at the sample size based on the analysis of two primary outcome measures: FMD and PWV. We fixed the α level to 0.05 and power at 80%. We used standard deviations for the two outcomes from the literature looking at patients with hyperuricemia (in the case of FMD) and at patients with chronic inflammatory conditions in the case of

Table I. Clinical and Demographic Characteristics: Bivariate Analysis

Variable	Control (n=20)	Gout (n=20)	*P* Value
Age, y	41.7±10.5	47.6±11	.19
Sex, No. (%)	17 (85)	19 (95)	.6
BMI	30.1±4.2	33.6±6.5	.054
SBP, mm Hg	140.7±11.2	138.6±18.4	.84
DBP, mm Hg	82.2±10.4	83.6±9.7	.52
Total cholesterol, mg/dL	187.6±32.0	200±27.4	.39
Triglycerides, mg/dL	97.9±39.5	201.3±153.0	**.001**
HDL, mg/dL	50.7±10.5	43.1±8.7	**.02**
LDL, mg/dL	116±26.8	122.0±24.5	.73
Serum uric acid, mg/dL	6.1±1.4	8.9±1.4	**<.0001**
Urine uric acid, 24-h	460±204.1	727.0±271.6	**.004**
BUN, mg/dL	14.9±3.6	16.2±3.2	.27
Creatinine, mg/dL	0.9±0.1	1.2±0.9	.14
Fasting glucose, mg/dL	91.4±10.9	91.3±10.0	.83
Framingham risk score	2.2±3.8	4.3±2.1	.053

Values are expressed as mean ± standard deviation unless otherwise indicated. Abbreviations: BMI, body mass index; BUN, serum urea nitrogen; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

Table II. Circulating Biomarkers of Vascular Function: Bivariate Analysis

Biomarkers	Control (n=20)	Gout (n=20)	*P* Value
CRP, mg/dL	0.2±0.16	1±2.3	**.01**
Tissue factor, pg/mL	20.9±16	27.7±14.5	.31
ICAM-1, pg/mL	169.2±34.4	188.3±53.6	.18
VCAM-1, pg/mL	504.6±100	575.5±174.6	.18
MCP-1, pg/mL	366.9±196	458.3±187.7	.14
IL-6, pg/mL	1.84±2.7	6.44±15	.34
PAI-1/TPA ratio	15.97±15.2	12.8±9.3	.7
Circulating apoptotic endothelial cells, cells/mL of blood	1429±2481.3	1407.8±2183.7	.37
Endothelial progenitor cells, cells/mL of blood	298.7±557.2	406.9±539.7	.24

Values are expressed as mean ± standard deviation unless otherwise indicated. Abbreviations: CRP, C-reactive protein; ICAM-1, intracellular adhesion molecule-1; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; TPA, tissue plasminogen activator; VCAM-1, vascular cellular adhesion molecule-1.

arterial compliance, since no studies were available from the literature that looked at gout and/or hyperuricemia and abnormal arterial compliance)[18,19] (FMD, 0.5% change; arterial compliance, 3.1 mL/mm Hg ×10). Based on these power calculations, we estimated that small to modest differences could be detected when compared with the standard deviation of these measures by studying 20 patients per group. A probability (*P*) value of .05 or smaller was considered significant for all hypotheses tests. No adjustment for multiple comparisons was performed. The above procedures were performed using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Demographic and Clinical Characteristics of Patient Populations

Overall, 20 patients with gout and 20 controls were studied. Bivariate analyses showed no significant differences in age, sex, or ethnicity between control and gout patients (Table I and not shown, respectively). The average disease duration since first gout diagnosis was 7.7 years (range, 1–25 years). A total of 10% of patients had tophi. The mean number of gout attacks per year was 4.4±0.8 (mean ± standard error of the mean [SEM]). The time range between last acute gout attack and time of visit was 0 to 24 months. Two of the patients were experiencing an acute gout attack at the time of the visit where vascular procedures were performed. A 70% of the patients were taking allopurinol at screening and underwent a month of drug washout before assessment of vascular function. No patients were taking uricosuric agents, adrenocorticotropic hormone, or febuxostat. A total of 35% of patients were taking daily oral prednisone, 35% were taking daily colchicine, and 90% were taking a nonsteroidal anti-inflammatory drug. These drugs were stopped 2 weeks prior to the vascular studies during the washout period. UA levels in the gout patients significantly increased after allopurinol washout, from 8±0.4 mg/dL to 9.2±0.23 mg/dL (mean ± SEM; $P<.01$). Two controls (10%) and 6 gout patients (30%) were taking antihypertensives at the time of the study. These included β-blockers, angiotensin-converting enzyme inhibitors, and thiazide monotherapy.

Bivariate analysis also showed patients with gout having, on average, a higher body mass index (BMI), significantly lower high-density lipoprotein (HDL) cholesterol, and higher triglycerides. There were no significant differences in arterial BP, total or low-density lipoprotein cholesterol, fasting glucose, serum creatinine, or BUN between both groups. As expected, patients with gout had significantly higher levels of serum and 24-hour urine UA (Table I). Despite the exclusion of smokers, patients requiring statins, and diabetics, patients with gout had overall higher Framingham risk score that approached statistical significance (Table I).

Most Biomarkers of Endothelial Damage Are Not Increased in Gout

Patients with gout had significantly higher circulating CRP levels than controls. While several of the biomarkers tended to be higher in the gout group, there were no statistically significant differences with controls for circulating levels of ICAM-1, VCAM-1, MCP-1, TF, IL-6, apoptotic endothelial cells, or EPCs. PAI-1/TPA ratios did not differ between groups in the bivariate analyses (Table II).

Vascular Function Is Not Impaired in Patients With Gout

Patients with gout had a significantly higher BAD (Table III and Table IV) ($P<.05$), which was associated with higher BMI (0.3; $P=.07$). There were no significant differences between groups in

Table III. Measurements of Vascular Function: Control Group

Biomarkers	Mean (n=20)	SD	IQR	Min	Max
Aortic SP, mm Hg	119.9	14.3	19.0	92.0	151.0
Pulse pressure, mm Hg	38.6	11.7	12.0	16.0	61.0
Base artery diameter, mm	4.5	0.98	1.12	2.7	7.1
Pulse wave velocity, cm sec^{-1}	8.2	1.6	1.5	5.0	11.7
Augmentation pressure, mm Hg	10.21	7.1	6.0	2.0	31.0
Reactive hyperemia index, %	2.08	0.4	0.5	1.1	2.8
FMD, %	4.2	9.5	9.95	−20.3	20.2
NMD, %	2.0	1.3	1.0	0.55	6.4
Augmentation index to 75 beats per min, %	16.9	9.3	11.0	3.0	34.0

Abbreviations: FMD, flow-mediated dilatation; IQR, interquartile range; max, maximum; min, minimum; NMD, nitroglycerin-mediated dilatation; SD, standard deviation; SP, systolic pressure.

Table IV. Measurements of Vascular Function: Gout Group

Biomarkers	Mean (n=20)	SD	IQR	Min	Max
Aortic SP, mm Hg	128.8	21.7	27.0	107.0	201.0
Pulse pressure, mm Hg	47.5	18.6	16.0	30.0	109.0
Base artery diameter, mm	5.1	0.92	1.2	3.1	6.6
Pulse wave velocity, cm sec^{-1}	8.9	1.6	1.6	6.6	13.6
Augmentation pressure, mm Hg	11.6	6.8	8.5	2.0	26.0
Reactive hyperemia index, %	2.3	0.6	0.97	1.3	3.6
FMD, %	5.3	11.8	7.8	−14.8	35.7
NMD, %	1.6	0.6	0.7	0.8	3.3
Augmentation index to 75 beats per min, %	17.1	10.3	16.5	1.0	37.0

Abbreviations: FMD, flow-mediated dilatation; IQR, interquartile range; max, maximum; min, minimum; NMD, nitroglycerin-mediated dilatation; SD, standard deviation; SP, systolic pressure.

conduit endothelial-dependent function, microvascular endothelial function, arterial compliance, VSM function, or any of the other vascular parameters measured (Table III, Table IV, and not shown, respectively). While not statistically significant, aortic systolic BP and pulse pressure were higher in the gout patients than in healthy controls (Table III and Table IV).

UA Does Not Determine Endothelial Function in Patients With Gout or Controls With Low CV Comorbidities

Performing correlation analysis in the control population, serum UA significantly correlated with diastolic BP (0.55; *P*=.01) and fasting glucose (0.59; *P*=.005), while levels of 24-hour urine UA did not correlate with any measurements (not shown). With a similar analysis in the gout patients, UA levels did not correlate with traditional risk factors of CVD, although there was a trend for a significant correlation with BMI (0.42; *P*=.06). Since the sample of gout patients studied had a significantly higher BMI than the controls, it is likely that this trend is due to the population difference.

UA levels did not predict any of the various vascular function measures in controls and gout patients. Similarly, there was no association between urine UA and any of the biomarkers in the gout and control groups (not shown).

Determinants of Vascular Function in Patients With Gout

When assessing variables that correlated with various vascular measurements, these varied among gout and control patients. In the control group, the main variables that significantly correlated with conduit or microvascular function were ICAM-1 (negative correlation with reactive hyperemia index; *P*=−0.43) and BMI (negative correlation with NMD; *P*=−0.5). Arterial stiffness (assessed by PWV) significantly correlated with diastolic BP (*P*=.5), while AIx@75 correlated with age (*P*=.49), HDL (*P*=.54), and systolic BP (*P*=.45). Aortic SP correlated with age, systolic BP, and ICAM-1 and VCAM-1 levels, while pulse pressure correlated with age and CRP.

In the gout group, the main variables that significantly correlated with conduit or microvascular

function were ICAM-1 (negative correlation with FMD; $P=-0.47$); diastolic BP (negative correlation with reactive hyperemia index; $P=-.44$), and tissue factor and PAI-1/TPA ratio (both showing positive correlation with NMD; $P=.85$ and $P=.9$, respectively). The main determinants of PWV in the gout group were age ($P=.73$) and diastolic BP ($P=.74$). AIx@75 showed a positive correlation with MCP-1 levels in the gout group ($P=.5$).

Multiple linear regression analysis was used to explore the predictive capability of circulating CV biomarkers on blood vessel function in both cohorts. When controlling only for group condition (gout and controls), ICAM-1 showed a negative significant correlation ($P=.03$) with FMD, indicating that individuals with higher levels of ICAM-1 are expected to have worse endothelial function, whether they have gout or not. ICAM-1 also continued to be a significant predictor of FMD in the gout patients ($P=.04$), controlling for all baseline clinical characteristics. Once all baseline variables were controlled, EPC numbers predicted FMD regardless of whether the patients were healthy or had gout ($P=.04$).

DISCUSSION

Our study assessed whether patients with gout and low traditional CV risk factors had evidence of conduit and microvascular endothelial dysfunction and arterial stiffness. Other additional confounders were eliminated, including the use of XO inhibitors, colchicine, and anti-inflammatories. UA correlated with diastolic BP and fasting glucose levels in the control group but showed no correlation with any of the demographic or clinical findings in the gout group. Similarly, in this group with low CV risk factors, UA levels did not predict or correlate with any of the conduit or microvascular function measurements in the gout or control patients. These observations were apparent despite the fact that patients with gout had higher Framingham risk score than the control group. These results appear to support the body of literature that indicates that, in those individuals with low CV risk and absence of other major comorbidities, UA is not a major predictor of vascular damage.[3,20]

The potential role of high UA level and gout in accelerated CVD is unclear. On one hand, epidemiologic studies have identified a strong relationship between hyperuricemia and/or gout and subsequent CV risk and mortality, particularly in high-risk groups.[21–23] While this correlation has been attributed to associations between UA and potential confounding factors, several studies suggest that the predictive power of hyperuricemia persists, even after considering these risk factors.[24] However, recent studies indicate that this enhanced risk is seen primarily in individuals already at risk for premature CVD, with a benign significance for high UA in individuals with low CV risk.[25] As such, in the general population and in patients at relatively low CV risk, serum urate has been at best a very weak predictor of vascular morbidity and mortality once effects of known confounders are considered.[25] In the Framingham study, hyperuricemia was not associated (after a 20-year follow-up) with increased CV risk,[26] and other large studies have reported similar findings.[4] The association of UA with coronary or carotid atherosclerosis is also controversial, given contradictory results among various ethnic populations and variations among sexes.[27,28] The epidemiologic data addressing an independent role of gout with CV events and mortality are also conflicting for similar reasons.[29,30] While differences in the methodologies used and the characteristics of the patient populations (age, sex, and comorbidities) have been proposed to justify these conflicting results, it remains unclear whether UA has a causal role in CV damage.

The confusion regarding UA's role in CVD promotion is enhanced by the various deleterious and homeostatic roles of this molecule in vascular biology. Hyperuricemia has potentially harmful effects on endothelial function, BP regulation, oxidative metabolism, and induction of proinflammatory molecules.[9,31,32] Allopurinol can decrease BP in hypertensive children,[33] and lowering UA improves metabolic syndrome in experimental conditions.[34] XO inhibition by allopurinol improves FMD,[35] but this action appears to be independent of UA reduction and the effects vary significantly among patient groups.[36–38] In addition, the hypothesis that UA alone induces endothelial dysfunction and that it can be reverted by its reduction, independently of the concomitant effects of XO on reactive oxygen species production, remains to be determined, as this enzyme induces free-radical synthesis under ischemia.[39]

Further against an independent role for hyperuricemia and/or gout in CVD, acute exposure to high concentrations of UA does not impair CV function in healthy men.[40] Enhancing vascular NO bioavailability by L-arginine supplementation causes a reduction in circulating UA;[41] therefore, UA may be responsive to vascular NO activity, consistent with a noncausal association between endothelial dysfunction and high UA.[36] UA is an important intracellular free-radical scavenger during metabolic

stress, and circulating concentrations are thought to be responsive to the local redox state. UA maintains various antioxidant systems by preventing inactivation of extracellular superoxide dismutase by hydrogen peroxide, the oxidation of ascorbate, and of tetrahydrobiopterin in cultured endothelial cells exposed to peroxynitrite.[42] Indeed, UA may account for 60% of the plasma antioxidant activity[43] and act as a compensatory antioxidant response. Despite the potential benefit of allopurinol in hypertensive kids,[33] adult patients taking allopurinol do not show consistent improvements in hypertension.[44] Other groups have proposed that hyperuricemia observed in patients with metabolic syndrome and other CV risk factors reflects a mechanism to neutralize high oxidative stress associated with insulin resistance and other comorbidities. Indeed, in patients with type 2 diabetes mellitus, lowering UA does not consistently improve endothelial function.[45] Overall, while animal models suggest that hyperuricemic animals develop endothelial dysfunction, human studies have given conflicting results.[31,32,46–48]

The apparent discrepancy between the association of UA and CVD in patients at low or high risk for vascular complications raises the possibility that hyperuricemia is of different significance depending on the person at risk, being a marker of ischemia and oxidative stress in patients at risk for CVD but not in individuals in which this risk is low overall. This appears to apply to otherwise healthy patients with chronic gout. Even though the gout patients studied had higher BMI and CRP levels and worse lipid parameters, the tested vascular function outcomes were comparatively preserved and did not differ from healthy individuals. While this may reflect in part a relatively small sample size or limitations or the study techniques, similar-sized protocols have observed abnormalities in one or more of these parameters in other diseases such as rheumatoid arthritis or systemic lupus erythematosus.[16,17] Patients with these diseases display subclinical vascular disease and dysfunction, even when traditional risk factors are accounted for.[12,13,16] These abnormalities contrast with the findings in this group of gout patients with low CV comorbidities, in which endothelial function and arterial compliance were overall preserved despite increased CRP levels.

It is worthy to note, however, that there were tendencies toward higher aortic systolic and pulse pressures among gout patients compared with controls. These increases appear to be larger than those observed in the arm systolic pressures and are not likely entirely explained by the minor brachial BP elevations. Perhaps this represents a finding of higher central aortic pressures in individuals with gout, which could independently increase CV risk, as previously reported for other patient populations.[49] As augmentation pressures were similar between groups, these systolic and pulse pressure differences are difficult to explain by an increased or earlier reflected PW. They might, however, be an early, more sensitive sign of increased arterial stiffness among gout patients than the PWV determination. These interesting findings merit some further consideration and represent the largest, albeit not significant, vascular differences between the groups. Patients with gout had increased baseline BAD, which correlated with increased BMI. Previous studies indicate that increased baseline BAD is associated with various conditions of enhanced CV risk, including BMI and glucose, and negatively correlates with HDL.[50,51] Men with increased basal BAD have significant increases in CV risk factors.[51] Large resting BAD has also been reported as an independent predictor of significant coronary artery disease in women with chest pain and is considered a potential atherosclerosis marker.[52] However, in the gout patients, BAD did not correlate with other CV risk factors or biomarkers and, in the absence of other abnormalities in vascular function, the relevance of this isolated finding is unclear. UA can upregulate angiotensinogen, angiotensin-converting enzyme, and angiotensin II receptors and lead to increased angiotensin II levels.[53] As such, future studies may also assess the potential correlation between UA, endothelial function and activation of the renin-angiotensin system in patients with gout.

In the study, the majority of variables that correlated with arterial stiffness and vascular function were traditional risk factors such as age and BP. In addition, soluble ICAM-1 showed a significant negative prediction with endothelial function, regardless of whether patients had gout or not. This is in line with a body of literature supporting ICAM-1 as a vascular damage biomarker and poor CV prognosis in various patient populations.[54,55]

A crucial factor to prevent CV damage and atherosclerosis is the maintenance of an adequate balance between endothelial cell destruction and repair. Various studies have indicated that decreased numbers and/or function of the bone marrow–derived EPCs is associated with enhanced CV risk and is present in various diseases associated with increased vascular damage. Our group and others have found that other chronic inflammatory conditions associated with enhanced vascular risk, including

rheumatoid arthritis and systemic lupus erythematosus, are characterized by enhanced endothelial damage not coupled by proper vascular repair.[17,56] In systemic lupus erythematosus, enhanced endothelial cell apoptosis strongly correlates with endothelial dysfunction, as assessed by brachial artery FMD.[16] While EPC function was not studied in this investigation, we did not find any evidence of lower circulating EPCs or increased apoptotic endothelial cells in gout patients with low Framingham risk factors. These findings do not exclude the possibility that the function of EPCs and/or other cells involved in vascular repair in gout may be impaired, and future studies should address this possibility. A transient surge in UA concentration has been proposed to herald tissue injury and accelerate EPCs' recruitment to vascular tissues,[57] but the effect of chronic hyperuricemia in the context of an inflammatory response, as seen in gout, remains unclear.

LIMITATIONS

This study has several limitations, including the study design, a small sample size, and the intrinsic variability expected from some of the vascular techniques used. The size of the study should be taken into account when interpreting the results. We planned to have 20 patients in each of our groups, based on estimates from the literature. Using the observed standard deviations, we were powered to detect differences of 9% in FMD and 1% in NMD. This means the true difference would need to be large before there would be statistical power to detect it. In addition, more gout patients than controls were taking antihypertensives and 10% of gout patients were experiencing a flare at the time of the study. Furthermore, a cross-sectional analysis of laboratory biomarkers of vascular damage poses limitations to the conclusions made from the study, given the known variability of some of these markers, including EPCs. These factors could have potentially altered the results.

While shown to be an independent predictor for the onset of CVD and a well-validated methodology used in numerous studies,[58] brachial FMD has some recognized limitations, including a moderate degree of inherent biological and technical variability.[59] FMD is a marker of NO-dependent vasodilatation; however, the method of upper-arm cuff occlusion we used, although valid, also provides some dilatation that is induced by other vasodilators.[59] It is possible that FMD differences between groups might have been detected if lower-arm cuff occlusion had been used, due to a higher percentage of NO-mediated vasodilatation. The major limitation of PAT determination through EndoPAT is the relatively limited number of studies demonstrating its independent association with hard CV outcomes. We used it as an adjunct to explore microvascular differences not found by using brachial FMD alone. Finally, studies using SphygmoCor devices have recently shown that the variables investigated provide independent prediction of CV outcomes.[60] Using all three methods together allowed us to explore for various potential sites of vascular dysfunction; however, it may have also increased the possibility for chance associations. Since no differences were found using these three different methods, we believe this enhances the likelihood that no minor vascular differences occurred between the healthy gout cohort and normal patients analyzed in this study.

CONCLUSIONS

Overall, the results from this study do not support the notion that gout is associated with vascular endothelial dysfunction in the conduit or microvasculature or to a significant increase in vascular damage biomarkers, in the absence of significant traditional CV risk factors. Despite the fact that gout patients were overall less healthy with regard to CV risk, these findings did not impact vascular function. At this point, whether hyperuricemia is a pathogenic factor in CV risk in the general population remains uncertain. Prospective randomized studies that target UA reduction may be necessary to address this controversy. Furthermore, assessing whether dual targeting of the anti-inflammatory cascade and hyperuricemia in gout leads to changes in vascular function may be warranted. However, our data do not support the notion that using drugs currently available to lower UA are justified in asymptomatic hyperuricemia to lower CV. Furthermore, the vascular dysfunction observed in other rheumatologic conditions such as rheumatoid arthritis is not observed in patients with gout.

Acknowledgments and disclosures: This study was supported by Takeda Pharmaceuticals through an investigator-initiated grant to MJK. The study was also funded in part by the NIH through CTSA grant UL1RR024986. We thank Ms Cynthia Harper for assistance in the preparation of the manuscript. Dr Kaplan received funding for this study from Takeda Pharmaceuticals (investigator-initiated trial).

Authors' contributions: RB: Supervised and planned performance of vascular function experiments, interpreted vascular function measurements and helped draft manuscript; SY: carried out all immunoassays; JM: performed statistical analysis and helped draft the manuscript; SK: performed statistical analysis and helped draft the manuscript; RH:

recruited patients and obtained data; SL: performed and analyzed vascular function measurements; WM: obtained clinical data; AN: data management; MJK: conceived and designed the study, recruited patients, obtained data, analyzed results and drafted the manuscript.

References

1 Baker JF, Krishnan E, Chen L, et al. Serum uric acid and cardiovascular disease: recent developments, and where do they leave us? *Am J Med*. 2005;118(8):816–826.
2 Hoieggen A, Alderman MH, Kjeldsen SE, et al. The impact of serum uric acid on cardiovascular outcomes in the LIFE study. *Kidney Int*. 2004;65(3):1041–1049.
3 Wen CP, David Cheng TY, Chan HT, et al. Is high serum uric acid a risk marker or a target for treatment? Examination of its independent effect in a large cohort with low cardiovascular risk. *Am J Kidney Dis*. 2010;56(2):273–288.
4 Iribarren C, Folsom AR, Eckfeldt JH, et al. Correlates of uric acid and its association with asymptomatic carotid atherosclerosis: the ARIC Study. Atherosclerosis risk in Communities. *Ann Epidemiol*. 1996;6(4):331–340.
5 Kim SY, Guevara JP, Kim KM, et al. Hyperuricemia and coronary heart disease: a systematic review and meta-analysis. *Arthritis Care Res (Hoboken)*. 2010;62(2):170–180.
6 Alderman MH. Podagra, uric acid, and cardiovascular disease. *Circulation*. 2007;116(8):880–883.
7 Boogaerts MA, Hammerschmidt DE, Roelant C, et al. Mechanisms of vascular damage in gout and oxalosis: crystal induced, granulocyte mediated, endothelial injury. *Thromb Haemost*. 1983;50(2):576–580.
8 Chapman PT, Yarwood H, Harrison AA, et al. Endothelial activation in monosodium urate monohydrate crystal-induced inflammation: in vitro and in vivo studies on the roles of tumor necrosis factor alpha and interleukin-1. *Arthritis Rheum*. 1997;40(5):955–965.
9 Kang DH, Park SK, Lee IK, et al. Uric acid-induced C-reactive protein expression: implication on cell proliferation and nitric oxide production of human vascular cells. *J Am Soc Nephrol*. 2005;16(12):3553–3562.
10 Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340(8828):1111–1115.
11 Wong M, Toh L, Wilson A, et al. Reduced arterial elasticity in rheumatoid arthritis and the relationship to vascular disease risk factors and inflammation. *Arthritis Rheum*. 2003;48(1):81–89.
12 Maki-Petaja KM, Hall FC, Booth AD, et al. Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-alpha therapy. *Circulation*. 2006;114(11):1185–1192.
13 Sari I, Okan T, Akar S, et al. Impaired endothelial function in patients with ankylosing spondylitis. *Rheumatology (Oxford)*. 2006;45(3):283–286.
14 Wallace SL, Robinson H, Masi AT, et al. Preliminary criteria for the classification of the acute arthritis of primary gout. *Arthritis Rheum*. 1977;20(3):895–900.
15 Brook RD, Brook JR, Urch B, et al. Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. *Circulation*. 2002;105(13):1534–1536.
16 Rajagopalan S, Somers EC, Brook RD, et al. Endothelial cell apoptosis in systemic lupus erythematosus: a common pathway for abnormal vascular function and thrombosis propensity. *Blood*. 2004;103(10):3677–3683.
17 Denny MF, Thacker S, Mehta H, et al. Interferon-alpha promotes abnormal vasculogenesis in lupus: a potential pathway for premature atherosclerosis. *Blood*. 2007;110(8):2907–2915.
18 Van Doornum S, McColl G, Jenkins A, et al. Screening for atherosclerosis in patients with rheumatoid arthritis: comparison of two in vivo tests of vascular function. *Arthritis Rheum*. 2003;48(1):72–80.
19 Erdogan D, Gullu H, Caliskan M, et al. Relationship of serum uric acid to measures of endothelial function and atherosclerosis in healthy adults. *Int J Clin Pract*. 2005;59(11):1276–1282.
20 Shah A, Keenan RT. Gout, hyperuricemia, and the risk of cardiovascular disease: cause and effect? *Curr Rheumatol Rep*. 2010;12(2):118–124.
21 Krishnan E, Svendsen K, Neaton JD, et al. Long-term cardiovascular mortality among middle-aged men with gout. *Arch Intern Med*. 2008;168(10):1104–1110.
22 Choi HK, Curhan G. Independent impact of gout on mortality and risk for coronary heart disease. *Circulation*. 2007;116(8):894–900.
23 Strasak A, Ruttmann E, Brant L, et al. Serum uric acid and risk of cardiovascular mortality: a prospective long-term study of 83,683 Austrian men. *Clin Chem*. 2008;54(2):273–284.
24 Corry DB, Tuck ML. Uric acid and the vasculature. *Curr Hypertens Rep*. 2006;8(2):116–119.
25 Johnson RJ, Kang DH, Feig D, et al. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? *Hypertension*. 2003;41(6):1183–1190.
26 Culleton BF, Larson MG, Kannel WB, Levy D. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. *Ann Intern Med*. 1999;131:7–13.
27 Tan TY, Lu CH, Lin TK, et al. Factors associated with gender difference in the intima-media thickness of the common carotid artery. *Clin Radiol*. 2009;64(11):1097–1103.
28 Neogi T, Ellison RC, Hunt S, et al. Serum uric acid is associated with carotid plaques: the National Heart, Lung, and Blood Institute Family Heart Study. *J Rheumatol*. 2009;36(2):378–384.
29 Abbott RD, Brand FN, Kannel WB, et al. Gout and coronary heart disease: the Framingham Study. *J Clin Epidemiol*. 1988;41(3):237–242.
30 De Muckadell OB, Gyntelberg F. Occurrence of gout in Copenhagen males aged 40–59. *Int J Epidemiol*. 1976;5(2):153–158.
31 Kanellis J, Kang DH. Uric acid as a mediator of endothelial dysfunction, inflammation, and vascular disease. *Semin Nephrol*. 2005;25(1):39–42.
32 Kato M, Hisatome I, Tomikura Y, et al. Status of endothelial dependent vasodilation in patients with hyperuricemia. *Am J Cardiol*. 2005;96(11):1576–1578.
33 Feig DI, Soletsky B, Johnson RJ. Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial. *JAMA*. 2008;300(8):924–932.
34 Nakagawa T, Tuttle KR, Short RA, et al. Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. *Nat Clin Pract*. 2005;1(2):80–86.
35 Doehner W, Schoene N, Rauchhaus M, et al. Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: results from 2 placebo-controlled studies. *Circulation*. 2002;105(22):2619–2624.
36 Eskurza I, Kahn ZD, Seals DR. Xanthine oxidase does not contribute to impaired peripheral conduit artery endothelium-dependent dilatation with ageing. *J Physiol*. 2006,571(Pt 3):661–668.
37 O'Driscoll JG, Green DJ, Rankin JM, et al. Nitric oxide-dependent endothelial function is unaffected by allopurinol in hypercholesterolaemic subjects. *Clin Exp Pharmacol Physiol*. 1999;26(10):779–783.

38 Butler R, Morris AD, Belch JJ, et al. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension*. 2000;35(3):746–751.
39 George J, Struthers AD. Role of urate, xanthine oxidase and the effects of allopurinol in vascular oxidative stress. *Vasc Health Risk Manag*. 2009;5(1):265–272.
40 Waring WS, Adwani SH, Breukels O, et al. Hyperuricaemia does not impair cardiovascular function in healthy adults. *Heart*. 2004;90(2):155–159.
41 Maxwell AJ, Bruinsma KA. Uric acid is closely linked to vascular nitric oxide activity. Evidence for mechanism of association with cardiovascular disease. *J Am Coll Cardiol*. 2001;38(7):1850–1858.
42 Ames BN, Cathcart R, Schwiers E, et al. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci USA*. 1981;78(11):6858–6862.
43 Nieto FJ, Iribarren C, Gross MD, et al. Uric acid and serum antioxidant capacity: a reaction to atherosclerosis? *Atherosclerosis*. 2000;148(1):131–139.
44 Vaccarino V, Krumholz HM. Risk factors for cardiovascular disease: one down, many more to evaluate. *Ann Intern Med*. 1999;131(1):62–63.
45 Waring WS, McKnight JA, Webb DJ, et al. Lowering serum urate does not improve endothelial function in patients with type 2 diabetes. *Diabetologia*. 2007;50(12):2572–2579.
46 Khan F, George J, Wong K, et al. The association between serum urate levels and arterial stiffness/endothelial function in stroke survivors. *Atherosclerosis*. 2008;200(2):374–379.
47 Mercuro G, Vitale C, Cerquetani E, et al. Effect of hyperuricemia upon endothelial function in patients at increased cardiovascular risk. *Am J Cardiol*. 2004;94(7):932–935.
48 Sakr SA, Abbas TM, Amer MZ, et al. Microvascular angina. The possible role of inflammation, uric acid, and endothelial dysfunction. *Int Heart J*. 2009;50(4):407–419.
49 Williams B, Lacy PS, Thom SM, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation*. 2006;113(9):1213–1225.
50 Jensen-Urstad K, Johansson J, Jensen-Urstad M. Vascular function correlates with risk factors for cardiovascular disease in a healthy population of 35-year-old subjects. *J Intern Med*. 1997;241(6):507–513.
51 Laclaustra M, Frangi AF, Frangi AG, et al. Association of endothelial function and vascular data with LDL-c and HDL-c in a homogeneous population of middle-aged, healthy military men: evidence for a critical role of optimal lipid levels. *Int J Cardiol*. 2008;125(3):376–382.
52 Holubkov R, Karas RH, Pepine CJ, et al. Large brachial artery diameter is associated with angiographic coronary artery disease in women. *Am Heart J*. 2002;143(5):802–807.
53 Yu MA, Sanchez-Lozada LG, Johnson RJ, et al. Oxidative stress with an activation of the renin-angiotensin system in human vascular endothelial cells as a novel mechanism of uric acid-induced endothelial dysfunction. *J Hypertens*. 2010;28(6):1234–1242.
54 Hartford M, Wiklund O, Mattsson Hulten L, et al. C-reactive protein, interleukin-6, secretory phospholipase A2 group IIA and intercellular adhesion molecule-1 in the prediction of late outcome events after acute coronary syndromes. *J Intern Med*. 2007;262(5):526–536.
55 Albert MA, Glynn RJ, Buring JE, et al. Differential effect of soluble intercellular adhesion molecule-1 on the progression of atherosclerosis as compared to arterial thrombosis: a prospective analysis of the Women's Health Study. *Atherosclerosis*. 2008;197(1):297–302.
56 Grisar J, Aletaha D, Steiner CW, et al. Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. *Circulation*. 2005;111(2):204–211.
57 Patschan D, Patschan S, Gobe GG, et al. Uric acid heralds ischemic tissue injury to mobilize endothelial progenitor cells. *J Am Soc Nephrol*. 2007;18(5):1516–1524.
58 Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. *Circulation*. 2009;120(6):502–509.
59 Harris RA, Nishiyama SK, Wray DW, et al. Ultrasound assessment of flow-mediated dilation. *Hypertension*. 2010;55(5):1075–1085.
60 Vlachopoulos C, Aznaouridis K, O'Rourke MF, et al. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J*. 2010;31(15):1865–1871.