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Clustering Patients With Gout Based on Comorbidities and Biomarkers: A Cross-Sectional Study

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ABSTRACT. ***Objective.*** This single-center clinical study identifies clusters of different phenotypes and pathophysiology subtypes of patients with gout and associated comorbidities.

Methods. Patients clinically diagnosed with gout were enrolled between January 2018 and December 2019. Hierarchical cluster analyses were performed using clinical data or biological markers, inflammatory markers, and oxidative stress pathway metabolites assayed from serum and plasma samples. Subgroup clusters were compared using ANOVA for continuous data and chi-square tests for categorical data.

Results. Hierarchical cluster analysis identified 3 clusters. Cluster 1 (C1; n = 24) comprised dyslipidemia, hypertension, and early-onset gout, without tophi. Cluster 2 (C2; n = 25) comprised hypertension, dyslipidemia, nephrolithiasis, and obesity. Cluster 3 (C3; n = 39) comprised multiple comorbidities and tophi. Post hoc comparisons of data obtained from samples of patients in C1, C2, and C3 revealed significant differences in the levels of oxidative stress and inflammation-related markers, including 3-nitrotyrosine, tumor necrosis factor, C-reactive protein, interleukin (IL) 1β, IL-6, platelet-derived growth factor (PDGF)–AA, and PDGF-BB. Reclustering patients based on all markers as well as on the biological markers that significantly differed among the initial clusters identified similar clusters.

Conclusion. Oxidative stress and inflammatory marker levels may affect the development and clinical manifestations (ie, clinical phenotypes) of gout. Measuring oxidative stress and levels of inflammatory cytokines is a potential adjunctive tool and biomarker for early identification and management of gout.

Key Indexing Terms: biomarkers, comorbidity, gout, hyperuricemia, uric acid

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Gout, the most common inflammatory arthritis, affects 3.9% of the adult population in the United States and is characterized by chronic inflammation and associated with hyperuricemia. Clinically, gout manifests as acute recurrent flares associated with increased oxidative stress and inflammatory cytokines.[1] Gout flares are potential risk factors for myocardial infarction,[2,3] type 2 diabetes mellitus (DM),[4] chronic kidney disease (CKD),[5] and premature mortality,[2,6,7] and may be followed by transient cardiovascular events, thereby exacerbating preexisting comorbidities and increasing the risk of new comorbidities.[8,9] In turn, these comorbidities can increase the risk of gout flares, therefore adversely affecting patients' quality of life.[10,11] Previous studies have described comorbidity clustering patterns in patients with gout in France,[12] Sweden,[13] the United Kingdom,[14] and Taiwan.[15] However, the underlying mechanisms of gout-associated comorbidities remain to be fully understood.

Several pathways have been identified to link gout-associated comorbidities with hyperuricemia, oxidative stress, and inflammation.[1,16] Further, serum urate (SU), a key pathophysiological abnormality in gout, is a potent antioxidant, primarily in the plasma[17]; and is a possible pro-oxidant in cells. The oxidation of hypoxanthine to uric acid catalyzed by xanthine oxidoreductase is a potential source for superoxide and hydrogen peroxide (ie, reactive oxygen species [ROS]).[16,18] Oxidative stress, induced by ROS or reactive nitrogen species, leads to the formation of lipid F2-isoprostanes, protein carbonyls, 3-nitrotyrosines, and thiols, and when these compounds accumulate, they cause cellular and organ dysfunction. Hyperuricemia is associated with inflammatory cytokine expression upregulation, which activates the renin-angiotensin-aldosterone system, increases C-reactive protein (CRP) and tumor necrosis factor (TNF), and decreases nitric oxide.[1]

Urate-lowering therapies (ULTs), such as allopurinol, febuxostat, and pegloticase, can reduce inflammation and oxidative stress, potentially reducing cardiovascular events and preserving kidney function.[1] However, recent randomized trials have reported no renal benefit from ULTs.[19,20] Therefore, hyperuricemia is not firmly established as a causal factor for CKD progression and warrants further investigation. Although previous studies have monitored and measured various markers to assess the roles of inflammatory and oxidative stress–dependent pathways in patients with gout, their influence on specific phenotypes remains unknown. Disease heterogeneity in patients with gout is attributed to pathophysiology or associated comorbidities, thereby influencing patients' response to therapies. Identifying important clinical clusters of patients with gout with distinct phenotypes can help to facilitate gout management and potentially prevent end-organ damage. Therefore, we have conducted a single-center cross-sectional study to identify distinct phenotype clusters and pathophysiological subtypes of patients with gout and gout-related comorbidities.

METHODS

Study design. We conducted a cross-sectional study of patients diagnosed with gout, lasting over 96 weeks. We used the Rheumatology Arthritis Database and Repository registry of the University of Alabama at Birmingham for patient recruitment and sample collection between January 2018 and December 2019. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Alabama at Birmingham (IRP-300004585); informed consent was obtained from all patients.

Study population. Adult patients diagnosed with gout were included, whereas those with systemic lupus erythematosus, rheumatoid arthritis, juvenile arthritis or psoriasis, or psoriatic arthritis were excluded.

Data collection. Demographic, clinical, and laboratory data collected during the clinic visit at the time of enrollment were retrieved from the electronic health record system. Demographic data included age, race, sex, current alcohol consumption, and current smoking status. Clinical data included BMI, age at the time of gout diagnosis (ie, disease duration), tophi, gout flares, ULT, number of gout flares in the last year (categorized as 0, 1-2, or > 2), ischemic heart disease (ie, coronary heart disease, myocardial infarction, or angina pectoris diagnosis), cerebrovascular disease (ie, stroke or transient ischemic attack diagnosis), dyslipidemia (ie, diagnosis or a filled prescription for a lipid-lowering medication for dyslipidemia), hypertension (HTN; ie, diagnosis or a filled prescription for an antihypertensive medication for HTN), DM (ie, diagnosis), or nephrolithiasis (ie, diagnosis), as an active condition for each patient during enrollment. The medications used to identify HTN and dyslipidemia included those primarily prescribed to treat these conditions. The anti-HTN medications included calcium channel blockers, diuretics, alpha blockers, vasodilators (ie, hydralazine), beta blockers, angiotensin-converting enzyme inhibitors (ACEI), and angiotensin II receptor blockers. The medications for dyslipidemia included statins, fenofibrate, ezetimibe, and gemfibrozil.

CKD was defined as per the guidelines of the Kidney Disease: Improving Global Outcomes and National Kidney Foundation,[21,22] as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 persisting for at least 3 months. Obesity, which was defined as BMI ≥ 30, was categorized based on the World Health Organization classification as class I (BMI 30-34.9), class II (BMI 35-39.9), or class III (BMI ≥ 40; morbid obesity)[23,24]; BMI is calculated as weight in kilograms divided by height in meters squared. Gout duration was considered as the time between the age at diagnosis and enrollment. Gout severity was assessed using the following variables during visits: (1) gout flare frequency > 2 in the year before enrollment, (2) tophaceous gout, and (3) current SU level > 6 mg/dL. Conversely, gout status was categorized as in remission (no gout flares in the last year), mild (1-2 gout flares/year), or severe (> 2 gout flares/year).

Laboratory data included SU, serum creatinine, and eGFR during or before study enrollment (ie, the most immediate prior value).

Blood sample collection and assessment of oxidative stress and inflammatory cytokines. Serum and plasma blood samples were collected during enrollment. The plasma levels of protein carbonyls and free 8-isoprostane were measured using ELISA kits (catalog nos. 516351 and 10005020, respectively; Cayman). We measured 3-nitrotyrosine using the OxiSelect nitrotyrosine ELISA kit (catalog No. STA-305, Cell Biolabs). Hemolysis markers (ie, oxyhemoglobin [oxyHb], methemoglobin [metHb], free heme, and total heme [oxyHb + metHb + free heme]) were measured using spectral deconvolution.[25] Nitrite and nitrate concentrations were measured using a high-pressure liquid chromatography system coupled with the Griess reaction (ENO-30 analyzer; Eicom).[26]

The inflammatory markers and components of the renin/angiotensin axis were assessed using commercially available kits and were validated using ELISA-based procedures. Renin concentration was measured in 2- to 6-fold diluted samples using the Quantikine ELISA kit (R&D Systems). Aldosterone was measured in undiluted or 2-fold diluted samples using the Parameter ELISA kit (R&D Systems). CRP, interleukin (IL) 1β, platelet-derived growth factor (PDGF)–AA and –BB, monocyte chemoattractant protein (CCL2/MCP), IL-6, and TNF were measured in 2-fold diluted samples using the Magnetic Luminex Multiplex ELISA (R&D Systems). Analyte concentrations were determined by fluorescence intensity using the Bio-Plex 200 System multiplex plate reader (Bio-Rad).

Statistical analysis. Hierarchical cluster analysis was performed using a set

of dissimilarities for the \(n\) objects. Initially, each object was assigned to a cluster, and the algorithm proceeded iteratively, joining the 2 most similar clusters at each stage until a single cluster remained.[27] At each stage, the cluster distances were recomputed using the Lance-Williams dissimilarity update formula as previously described.[27] The Ward minimum variance method identified compact spherical clusters. In the hierarchical cluster display, specifying the subtree assigned to the left and right at each merge was required.[28] \(n\) observations had \(n–1\) merges and \(2^\) possible orderings for the leaves in a cluster tree or dendrogram. Therefore, the hclust algorithm ordered the subtree, such that the tighter cluster was on the left: the most recent merge of the left subtree was lower than that of the right subtree. Single observations formed the tightest clusters possible, while merges involving 2 observations were placed according to their observation sequence number.[27]

In total, 3 unsupervised cluster analyses of patients with gout were performed. First, clinical and comorbidity data were used (ie, demographics, gout history, comorbidity, and lifestyle habit variables); second, the biological marker data were used (ie, 3-nitrotyrosine, oxyHb, nitrate, TNF, IL-6, and CRP), with significantly different levels among initial clusters; and third, all biological markers were used.

The relationship among variables was analyzed by cluster analysis using the ClustOfVar package in R (version 3.5.2; R Foundation for Statistical Computing). Statistical analyses were performed using SPSS Statistics (version 27; IBM Corp). Graphs were plotted using Prism 9 software (GraphPad). Data were expressed as mean and SD or median and range. Descriptive categorical data were expressed as frequencies and percentages. All tests were 2-tailed, and statistical significance was set at $P < 0.05$.

RESULTS

Study population. Our study included 88 patients diagnosed with gout who were enrolled between January 2018 and December 2019. Patient characteristics, gout status, and comorbidities are listed in Table 1.

Unsupervised clustering of patients with gout based on clinical data. In total, 3 clusters of patients were identified (Figure 1 and Table 2). Cluster 1 (C1; 24/88, 27%) comprised dyslipidemia, HTN, and early gout onset, without tophi. Cluster 2 (C2; 25/88, 28%) comprised HTN, dyslipidemia, nephrolithiasis, and obesity. Cluster 3 (C3; 39/88, 44%) included multiple comorbidities and tophi. C1, C2, and C3 included 100%, 92%, and 46% male patients, respectively. The mean SU levels of C2 (7.74 mg/dL) were higher than those of C1 (6.83 mg/dL) and C3 (5.67 mg/dL). The mean age of patients in C3 at the time of gout diagnosis (52.31 yrs) was higher than that of patients in C1 (40.71 years) and C2 (48.76 years). The mean gout duration was highest in C1 patients (18.63 years), followed by C2 (14.84 years) and C3 (8.59 years) patients.

Compared with C2 and C3, C1 patients had high 3-nitrotyrosine oxyHb, total heme, CRP, TNF, IL-1β, PDGF-AA, and PDGF-BB (Figure 2; Supplementary Figure S1, available with the online version of this article; and Table 3), possibly because of long-standing gout or the associated comorbidities. Meanwhile, C2 patients had higher levels of nitrate, total protein, metHb, heme, renin, and aldosterone than C1 and C3 patients, which is suggestive of elevated oxidative stress markers and hemolysis possibly linked to renal impairment and multiple comorbidities. C3 patients had higher carbonyl, nitrite, 8-isoprostane, IL-6, and CCL2/MCP than C1 and C2 patients, indicating differences in oxidative stress and inflammatory cytokines related to lipid oxidation.

Reclustering of patients with gout based on 6 biological markers. Patients were reclustered based on 3 oxidative stress markers (ie, 3-nitrotyrosine, oxyHb, and nitrate) and 3 inflammatory cytokines (ie, TNF, IL-6, and CRP), which significantly differed among the 3 initial clusters (ie, C1, C2, and C3). The patient characteristics of these new clusters (ie, C*1, C*2, and C*3) are listed in Supplementary Table S1 (available with the online version of this article), while the baseline oxidative stress markers and inflammatory cytokines are shown in Figure 3 and in Supplementary Figure S2 and Table S2 (available with the online version of this article).

Patients in C*3 were older (52.40 yrs) at the time of diagnosis than those in C*1 (46.23 yrs) and C*2 (48.33 yrs). C*2 patients had higher mean serum creatinine (1.82 mg/dL) than patients in the other clusters. Compared with C*2 and C*3, C*1 patients had higher total protein, 3-nitrotyrosine, oxyHb, metHb, heme, total heme, CRP, TNF, IL-1β, PDGF-AA, renin, and CCL2/MCP (Figure 3 and Supplementary Table S2, available with the online version of this article). Nitrate and aldosterone were higher in C*2 patients than in C*1 and C*3 patients. Nitrite, 8-isoprostane, carbonyl, IL-6, and PDGF-BB were higher in C*3 patients than in C*1 and C*2 patients (Figure 3 and Supplementary Table S2).

Reclustering of patients with gout based on all biological markers. Reclustering patients based on all biological markers led to 3 significantly distinguished clusters, namely C**1, C**2, and C**3. C**1 (36/88, 41%), C**2 (16/88, 18%), and C**3 (36/88, 41%) comprised 92%, 69%, and 58% male patients, respectively (Supplementary Table S3, available with the online version of this article). Patients in C**1 were younger at the time of diagnosis, with a longer mean duration of gout than those in C**2 and C**3. MetHb, 3-nitrotyrosine, TNF, CRP, and PDGF-AA were higher in C**1 patients than in C**2 and C**3 patients (Supplementary Figure S3 and Table S4).

The total protein, heme, total heme, aldosterone, renin, IL-1β, CCL2/MCP, and PDGF-BB were higher in C**2 patients than in C**1 and C**3 patients (Supplementary Figure S3 and Table S4, available with the online version of this article).

Patients in C**3 were primarily of African American descent (81%) and were younger at enrollment (60.33 years) than those in C**1 and C**2 (61.56 years and 62.56 years, respectively). Carbonyl, nitrite, nitrate, 8-isoprostane, and IL-6 were higher in C**3 patients than in patients from the other clusters (Supplementary Figure S3 and Table S4, available with the online version of this article).

DISCUSSION

In this study, we demonstrated that gout patient clusters based on disease onset/severity and comorbidities differed by inflammatory and oxidative stress marker signatures. Our study confirms previous findings of systemic oxidative stress in patients with gout who had hyperuricemia, CKD, coronary artery disease, or cerebrovascular disease.[29,30]

Based on our observations in this study, we propose a theoretical model for gout and comorbidity comprising the interrelationship of 3 gout immunopathogenic layers, including

Table 1. Clinical characteristics of patients diagnosed with gout at the time of enrollment.

	All Patients, N = 88	ULT+, n = 71	ULT–, n = 17	*P*
Age at time of enrollment, yrs, mean (SD)	61.24 (11.94)	61.21 (12.52)	61.35 (8.78)	0.96
Age ≤ 65 yrs at time of enrollment	54 (61)	43 (61)	11 (65)	0.11
Age at time of gout diagnosis, yrs, mean (SD)	48.14 (14.31)	49.11 (13.94)	44.06 (15.07)	0.19
Disease duration, yrs, mean (SD)	13.10 (11.65)	12.10 (11.11)	17.29 (12.84)	0.10
Race/ethnicity				0.45
White	43 (49)	32 (45)	11 (65)	
African American	41 (47)	35 (49)	6 (35)	
Asian	3 (3)	3 (4)	0 (0)	
Hispanic or Latino	1 (1)	1 (1)	0 (0)	
Male sex	65 (74)	52 (73)	13 (76)	> 0.99
Current alcohol consumption	38 (43)	32 (45)	6 (35)	0.59
Smoking status				0.58
Never	59 (67)	46 (65)	13 (76)	
Former	18 (20)	15 (21)	3 (18)	
Current	11 (13)	10 (14)	1 (6)	
BMI[a], mean (SD)	34.32 (7.30)	34.77 (7.69)	32.44 (4.92)	0.24
BMI > 30	52 (59)	44 (62)	8 (47)	0.23
Tophaceous gout	14 (16)	13 (18)	1 (6)	0.21
No. of gout flares in the last year, mean (SD)	2.73 (3.71)	2.61 (2.38)	3.24 (6.88)	0.53
No. of gout flares in the last year				0.27
0	23 (26)	16 (22)	7 (41)	
1-2	29 (33)	24 (34)	5 (29)	
> 2	36 (41)	31 (44)	5 (29)	
Currently experiencing a gout flare	8 (9)	5 (7)	3 (18)	0.18
Comorbidity				
Hypertension	73 (83)	59 (83)	14 (82)	> 0.99
Dyslipidemia	53 (60)	44 (62)	9 (53)	> 0.99
Diabetes	31 (35)	25 (35)	6 (35)	> 0.99
Chronic kidney disease	32 (36)	27 (38)	5 (29)	0.58
Nephrolithiasis	19 (22)	13 (18)	6 (35)	0.19
Ischemic heart disease	16 (18)	14 (20)	2 (12)	0.73
Cardiovascular disease	6 (7)	5 (7)	1 (6)	> 0.99
ACR-EULAR gout classification criteria score, mean (SD)	10.42 (0.20)	10.38 (2.06)	10.59 (2.70)	0.73
ACR-EULAR gout classification criteria score ≥ 8	86 (98)	70 (99)	16 (94)	0.26
Current gout medication				< 0.001
Colchicine	28 (32)	28 (39)	0 (0)	
Allopurinol	48 (55)	48 (68)	0 (0)	
Febuxostat	8 (9)	8 (11)	0 (0)	
Probenecid	1 (1)	1 (1)	0 (0)	
Lesinurad	1 (1)	1 (1)	0 (0)	
Pegloticase	1 (1)	1 (1)	0 (0)	
Cherry extract	4 (5)	4 (6)	0 (0)	
Serum urate, mg/dL, mean (SD)	6.57 (2.15)	6.24 (2.11)	7.96 (1.73)	0.003
Serum urate > 6 mg/dL	38 (43)	25 (35)	13 (76)	
Serum creatinine, mg/dL, mean (SD)	1.66 (3.01)	1.66 (3.19)	1.66 (2.17)	0.99
Serum creatinine > 1.2 mg/dL	27 (31)	23 (32)	4 (24)	
eGFR, mL/min/1.73 m^2, mean (SD)	53.65 (13.18)	53.26 (13.10)	55.24 (13.37)	0.58
eGFR ≤ 60 mL/min/1.73 m^2	30 (34)	25 (35)	5 (29)	

Data are in n (%) unless otherwise indicated. [a] BMI is calculated as weight in kilograms divided by height in meters squared. ACR: American College of Rheumatology; eGFR: estimated glomerular filtration rate; EULAR: European Alliance of Associations for Rheumatology; ULT: urate-lowering therapy.

circulating biomarkers (ie, SU, oxidative stress, and inflammatory cytokines) and comorbidities (Supplementary Figure S4, available with the online version of this article). This multilayer model accounts for interactions among serum biomarkers that may induce clinical symptoms of gout, which may be associated with specific comorbidities. This framework can be evaluated with longitudinal studies of patients with gout exhibiting clinical symptoms and further modified as new insights are gained.

Herein, 3 clusters reflecting different phenotypes and pathophysiology subtypes of patients with gout were identified, namely

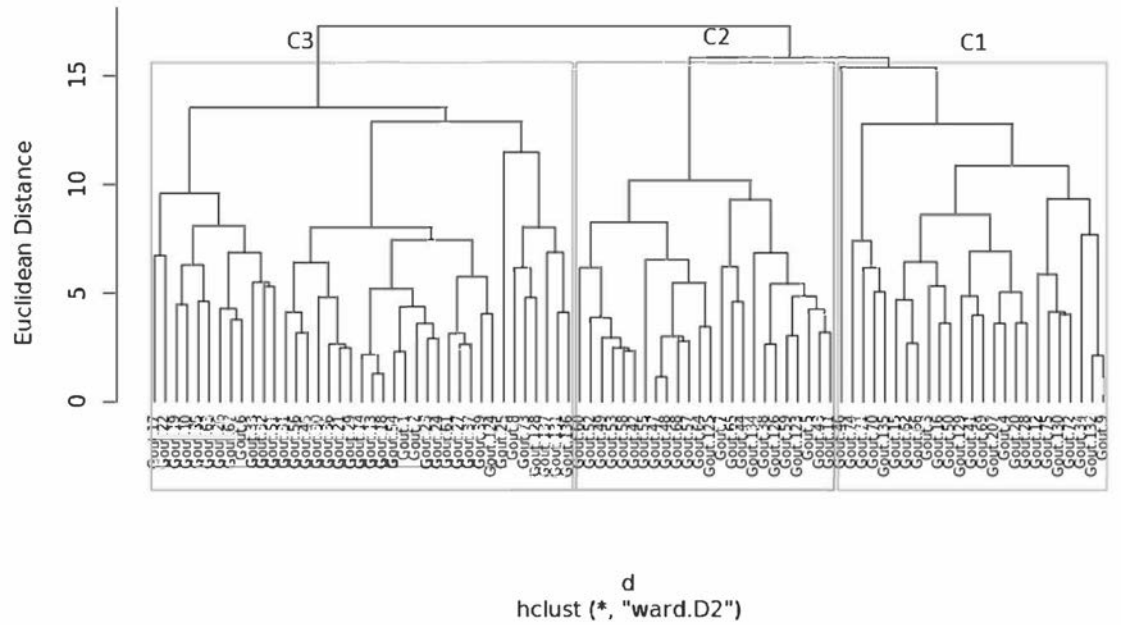

Figure 1. Dendrogram illustrating the results of cluster analysis based on comorbidity data (N = 88). The vertical axis represents the distance between clusters (Euclidean distance), while the horizontal axis represents the observations and clusters. Each vertical bar represents a subject and a cluster; the joining of 2 clusters is represented by the fusion of 2 vertical bars. The vertical position of the fusion, represented by short horizontal lines, indicates the distance between clusters. C1: cluster 1; C2: cluster 2; C3: cluster 3.

C1, C2, and C3, of which C2 included mostly White and obese patients with high serum creatinine and SU, in which less than half were on ULTs. Patients in C2 had higher MetHb and heme, with an activated renin-angiotensin system and higher aldosterone and renin compared with those in C1 and C3. C3 included almost equal proportions of male and female patients with gout and those with tophaceous gout and morbid obesity, mostly of African American descent. Patients in C3 showed higher oxidative stress markers (ie, carbonyl, nitrite, and 8-isoprostane) and inflammatory cytokines (ie, IL-6 and CCL2/MCP) compared with those in C1 and C2.

Previous studies have attempted to cluster patients with gout into subgroups with different comorbidities and, to this end, suggested an isolated cluster of gout without comorbidities.[12-15] This discrepancy could be attributed to differences in sampling between previous studies and our study. Our cohort was assembled from a rheumatology clinic, which provides care to patients with a more severe gout phenotype and with comorbidities compared with a primary care clinic. Our study sample was not a representative population.

Further, previous studies were conducted in Europe or Taiwan among patients with different genetic and nongenetic factors (ie, lifestyle habits, diet, and environment).[15,31] Nevertheless, our study identified a subgroup/cluster similar to that of previous studies.[12-15] For instance, in C1, the phenotypes dyslipidemia, HTN, and early gout onset, without tophi, were similar to "cluster 4" in a study by Bevis et al,[14] the "obesity and dyslipidemia" cluster reported by Fatima et al,[13] and the "obesity" cluster identified by Richette et al.[12] Similarly, C2 in this study, comprising patients with HTN, dyslipidemia, nephrolithiasis, and obesity, corresponded to the "CKD/kidney dysfunction" cluster, "cardio-metabolic disease" cluster, and "cardiorenal and diuretic" cluster identified in the studies by Fatima et al,[13] Bevis et al,[14] and Richette et al,[12] respectively. C3 comprised multiple comorbidities and tophi, corresponding to the "DM/HTN" cluster reported by Fatima et al[13] and the "dyslipidemia" cluster reported by Richette et al.[12]

To the best of our knowledge, our study is among the limited number of US studies consistent with the findings of previous cluster studies and the first to describe the association with inflammatory and oxidative stress markers in these clusters. These findings provide insights into population differences between this study and previous studies, as well as insights into the significance of sociodemographic and racial/ethnic differences in gout pathogenesis, comorbidity load, and patient outcomes.

We performed reclustering of patients diagnosed with gout based on cluster-dependent differences in 6 inflammatory and oxidative stress markers and all biological markers without consideration of clinical features. The clustering identified 3 clusters using both approaches. The identified clusters had the following similarities: 3-nitrotyrosine, TNF, CRP, IL-1β, and PDGF-AA were high in C1, C*1, and C**1; aldosterone was high in C2, C*2, and C**2; and carbonyl, nitrite, 8-isoprostane, and IL-6 were high in C3, C*3, and C**3. These findings support the robustness of our approach and suggest that specific oxidative stress and cytokine pathways are associated with, and can serve as surrogates to, the identified gout clinical clusters.

High oxidative stress and cytokine markers observed in C3 may be related to the dominant processes in tophaceous gout and obesity. Monosodium urate crystals in patients with gout activate toll-like receptors and engage the caspase-1–activating NALP3 inflammasome, leading to IL-1β, TNF, IL-18, and

Table 2. Clinical characteristics of patients diagnosed with gout in the 3 clusters based on comorbidities.

	Cluster 1, n = 24	Cluster 2, n = 25	Cluster 3, n = 39
Patients, N = 88	24 (27)	25 (28)	39 (44)
Demographics			
Male sex	24 (100)	23 (92)	18 (46)
White race	19 (79)	15 (60)	9 (23)
BMI[a], mean (SD)	29.70 (4.12)	32.96 (5.47)	38.03 (7.91)
Obese, with BMI ≥ 30	12 (50)	15 (60)	32 (82)
Lifestyle			
Smoking (current)	1 (4)	5 (20)	5 (13)
Smoking (former)	3 (13)	4 (16)	11 (28)
Current alcohol consumption	14 (58)	12 (48)	12 (31)
Gout			
Age at time of enrollment, yrs, mean (SD)	59.33 (12.31)	63.60 (10.19)	60.90 (12.48)
Age at time of diagnosis, yrs, mean (SD)	40.71 (13.92)	48.76 (13.56)	52.31 (13.16)
Duration, yrs, mean (SD)	18.63 (12.90)	14.84 (12.67)	8.59 (7.72)
ACR criteria score for gout diagnosis, mean (SD)	10.75 (2.31)	10.04 (1.99)	10.46 (2.23)
Individuals with gout flares in the last year	15 (63)	22 (88)	28 (72)
Gout flares (current)	1 (4)	0 (0)	7 (18)
Tophi, yes	0 (0)	5 (20)	9 (23)
Clinical laboratory findings			
Serum creatinine, mg/dL, mean (SD)	1.06 (0.22)	2.86 (5.36)	1.21 (0.51)
eGFR, mL/min/1.73 m^2, mean (SD)	59.50 (4.36)	47.80 (17.68)	54.18 (11.42)
Serum urate, mg/dL, mean (SD)	6.83 (1.90)	7.74 (2.22)	5.67 (1.84)
Comorbidity			
Diabetes	0 (0)	12 (48)	19 (49)
Hypertension	13 (54)	25 (100)	35 (90)
Ischemic heart disease	1 (4)	11 (44)	4 (10)
Cerebrovascular disease	0 (0)	5 (20)	1 (3)
Chronic kidney disease	3 (13)	14 (56)	13 (33)
Nephrolithiasis	3 (13)	16 (64)	0 (0)
Dyslipidemia	16 (67)	17 (68)	20 (51)
Current gout medication			
Colchicine	3 (13)	12 (48)	13 (33)
Allopurinol	13 (54)	8 (32)	27 (69)
Febuxostat	1 (4)	2 (8)	5 (13)
Pegloticase	0 (0)	1 (4)	0 (0)

Data are in n (%) unless otherwise indicated. [a] BMI is calculated as weight in kilograms divided by height in meters squared. ACR: American College of Rheumatology; eGFR: estimated glomerular filtration rate.

ROS secretion.[32] ROS damages macromolecules, such as lipids, proteins, complex carbohydrates, and nucleic acids, causing oxidative injury.[33,34] Obesity, which causes chronic low-grade inflammation, is another important source of oxidative stress in patients with gout.[35] Obesity induces systemic oxidative stress through multiple biochemical mechanisms, such as nicotinamide adenine dinucleotide phosphate oxidase superoxide generation, oxidative phosphorylation, glyceraldehyde auto-oxidation, protein kinase C activation, and the polyol and hexosamine pathways.[36,37] A significant positive correlation exists between BMI and oxidative stress biomarkers.[38] Increased IL-6, 8-isoprostane, and protein carbonylation has been reported in patients with obesity,[34,35] consistent with the findings of our study.

Previous studies involving patients with HTN and hyperlipidemia have revealed that 3-nitrotyrosine, TNF, CRP, IL-1β, and PDGF-AA significantly affect the pathogenesis of HTN.[39-43] Moreover, patients with essential HTN show high serum IL-1 levels, highlighting the prohypertensive effects of IL-1.[44] In contrast, the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) revealed that IL-1 might not be associated with HTN, as HTN was not observed in patients treated with the IL-1 inhibitor, canakinumab.[45] In our study, male patients in C1 presented hyperlipidemia, HTN, early-onset nontophaceous gout, and elevated levels of 3-nitrotyrosine, TNF, CRP, IL-1β, and PDGF-AA; however, their alcohol consumption rates were high. Alcohol consumption increases ROS production and reactive nitrogen species, thereby exacerbating nitro-oxidative stress and mitochondrial dysfunction, which ultimately promotes tissue injury.[46,47] Further, TNF, IL-1β, and IL-6 are elevated in alcohol-induced liver disease.[48] Hence, although we observed increased TNF, IL-1β, and IL-6 in some patients, their liver disease status was unknown, preventing further interpretation of these findings.

Identifying oxidative stress and inflammatory states in the 3 clusters of gout can effectively facilitate the management of gout and its associated comorbidities. High oxidative stress

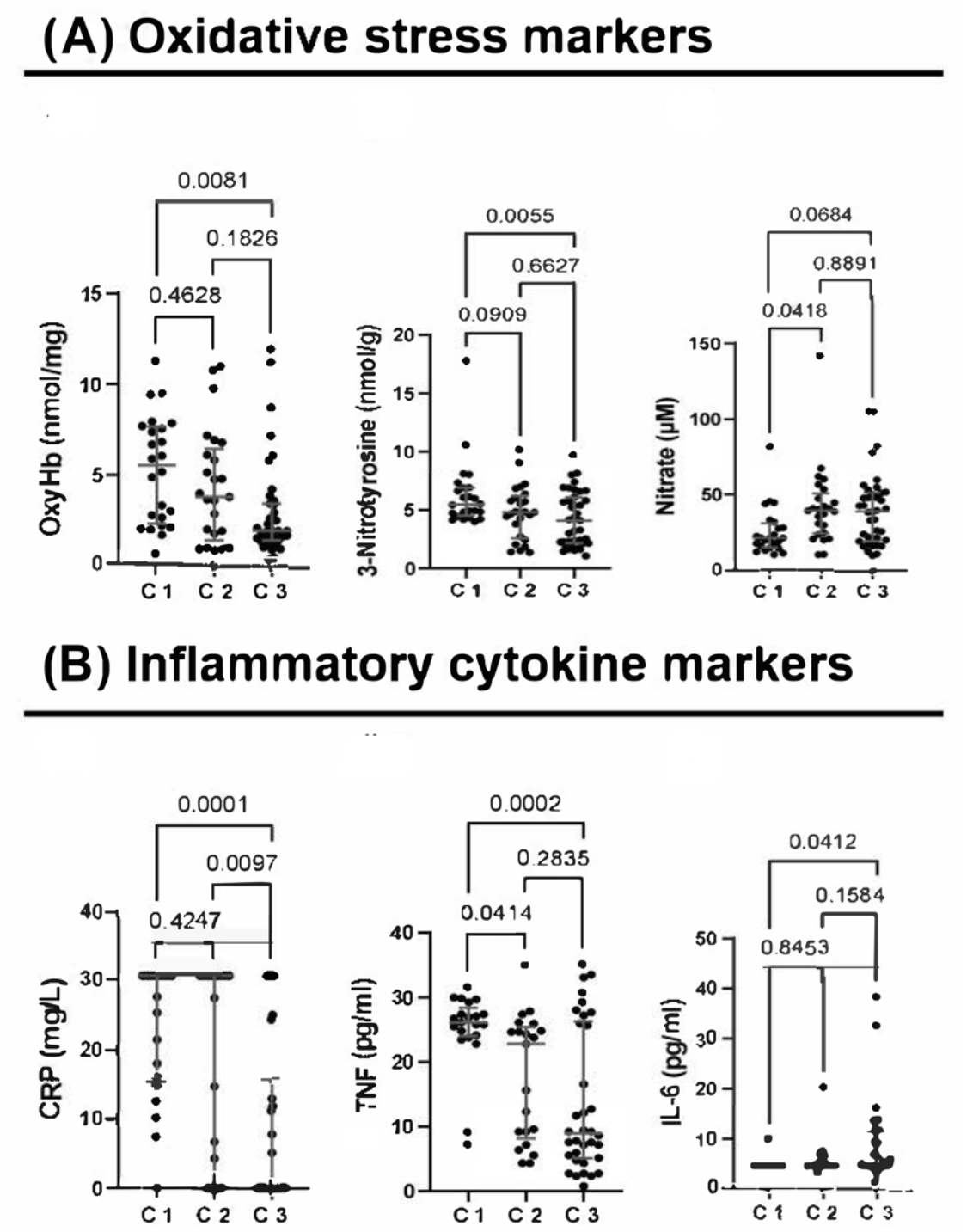

Figure 2. Biological markers in clusters 1 to 3 based on comorbidities. (A) Oxidative stress markers and (B) inflammatory cytokines. The markers were compared using ANOVA. *P* values above each panel indicate the significance between the test groups. C1: cluster 1; C2: cluster 2; C3: cluster 3; CRP: C-reactive protein; IL-6: interleukin 6; oxyHb: oxyhemoglobin; TNF; tumor necrosis factor.

induces mitochondrial dysregulation and inflammation, potentially contributing to endothelial dysfunction and increasing or worsening cardiovascular disease, renal fibrosis, atherosclerosis, and neurological disorder risks.[49] Allopurinol, a ULT frequently prescribed to patients with gout, exerts an antioxidant effect by inhibiting the xanthine oxidase–dependent production of nitric oxide and ROS in patients with hyperuricemic CKD.[30,50] Few studies have previously reported the prospective implications of comorbidity clustering for gout prognosis and comorbidity development.[15,32]

Bajpai et al[31] conducted a 5-year follow-up study on patients diagnosed with gout and retrieved their medical history from primary care records in 20 general practices. The authors found that new comorbidity development in these patients was

Table 3. Levels of oxidative stress markers and inflammatory cytokines in the 3 gout-related comorbidity clusters.

Marker	All, N = 88	Cluster 1, n = 24	Cluster 2, n = 25	Cluster 3, n = 39
Oxidative stress marker[a]				
OxyHb, μM	3.96 (3.03)	5.27 (2.91)	4.28 (3.10)	2.95 (2.68)
3-nitrotyrosine, nmol/g	4.97 (2.60)	6.32 (2.86)	4.80 (2.27)	4.24 (2.30)
Nitrate, μM	36.17 (23.43)	25.67 (15.47)	41.76 (25.47)	39.05 (24.06)
Total protein, mg/mL	52.11 (9.24)	52.05 (7.45)	53.54 (8.44)	51.24 (10.52)
Carbonyl content, nmol/mg	4.11 (5.56)	3.83 (4.78)	3.09 (4.11)	4.93 (6.60)
Nitrite, μM	0.22 (0.29)	0.12 (0.29)	0.20 (0.28)	0.30 (0.28)
8-isoprostane, pg/mL	11.50 (6.65)	9.45 (2.57)	10.53 (4.06)	13.37 (8.86)
Total heme, μM	28.25 (15.62)	30.09 (16.24)	29.91 (14.56)	26.06 (15.62)
MetHb, μM	0.35 (1.23)	0.35 (1.06)	0.53 (1.77)	0.24 (0.82)
Inflammatory cytokine marker[a]				
CRP, mg/L	15.38 (13.71)	23.44 (9.43)	18.67 (14.01)	8.37 (12.18)
TNF, pg/mL	17.90 (10.50)	25.01 (5.91)	17.70 (9.45)	13.64 (10.96)
IL-6, pg/mL	6.87 (5.70)	4.91 (1.14)	5.86 (3.37)	8.72 (7.64)
CCL2/MCP, pg/mL	358.89 (563.13)	290.95 (89.29)	326.42 (96.54)	420.91 (831.16)
IL-1β, pg/mL	13.59 (7.15)	16.23 (3.57)	13.29 (6.35)	12.14 (8.66)
Renin, pg/mL	2562.26 (5134.50)	1074.28 (1094.86)	3840.27 (6375.03)	2728.51 (5585.69)
Aldosterone, pg/mL	242.63 (223.46)	161.89 (59.06)	284.73 (284.78)	266.01 (231.55)
PDGF-AA, pg/mL	1186.86 (727.00)	1424.11 (684.18)	1099.31 (775.89)	1094.40 (687.34)
PDGF-BB, pg/mL	6735.87 (4095.09)	7945.87 (3712.73)	6239.65 (3429.24)	6295.00 (4522.99)

Data are in mean (SD). [a]Biomarker assessment: oxidative stress marker and inflammatory cytokine marker assays were performed for all patients. CRP: C-reactive protein; IL: interleukin; MCP: monocyte chemoattractant protein; MetHb: methemoglobin; OxyHb: oxyhemoglobin; PDGF: platelet-derived growth factor; TNF: tumor necrosis factor.

associated with their baseline comorbidity cluster. However, a higher incidence of gout flares was observed over time in participants not correlating with their baseline comorbidity cluster. Meanwhile, Huang et al[15] analyzed the longitudinal transition of patients with gout in the Taiwan Longitudinal Health Insurance Database and revealed that some patients experienced a longitudinal transition between low or moderate to high comorbidities. This effect was more prominent in older patients.

Our study findings supported the potential involvement of oxidative stress and inflammatory cytokine pathways in the development and clinical manifestations of gout and associated comorbidities. The strengths of our study include assessments of a comprehensive list of oxidative stress markers, inflammatory cytokines, and the renin-angiotensin system components in patients with gout.

Our study has some limitations, such as its cross-sectional design and use of specific clinical information, as laboratory (ie, creatinine level and eGFR) data were unavailable for some patients. Having a small sample size with a lack of power may have resulted in our study missing some associations. We did not adjust the data for multiple comparisons (ie, some study findings may have occurred because of chance). Additionally, lacking data from healthy volunteers (ie, controls) limited the interpretability of the results for oxidative and inflammatory cytokines. However, our aim was not to determine whether patients with gout differed from those without gout but, instead, to determine the differential phenotypes based on the levels of clinical or biochemical markers among patients with gout. Further, anti-HTN medications, such as ACEI, can affect SU, which may have influenced the results. Missing data also prevented the merging of certain comorbidities into metabolic syndrome. Further, we could not control the use of over-the-counter antioxidant supplements, such as omega-3 fatty acids and vitamin E, which might also have influenced our results. Therefore, validation studies with a larger sample size remain warranted.

In conclusion, this single-center, clinic-based, cross-sectional study identified 3 clusters of gout-associated comorbidities. Our results suggest that oxidative stress and inflammatory cytokine levels may affect the clinical outcomes of patients with gout and associated comorbidities. Therefore, mitigating oxidative stress and inflammatory cytokine levels could be a valuable adjunctive strategy for the effective management of difficult-to-treat gout. Our findings provide novel insights into, and expand our understanding of, the pathogenesis of gout and gout-associated comorbidities. Further research remains warranted to explore the pathophysiology of oxidative stress markers and inflammatory cytokines in these patients.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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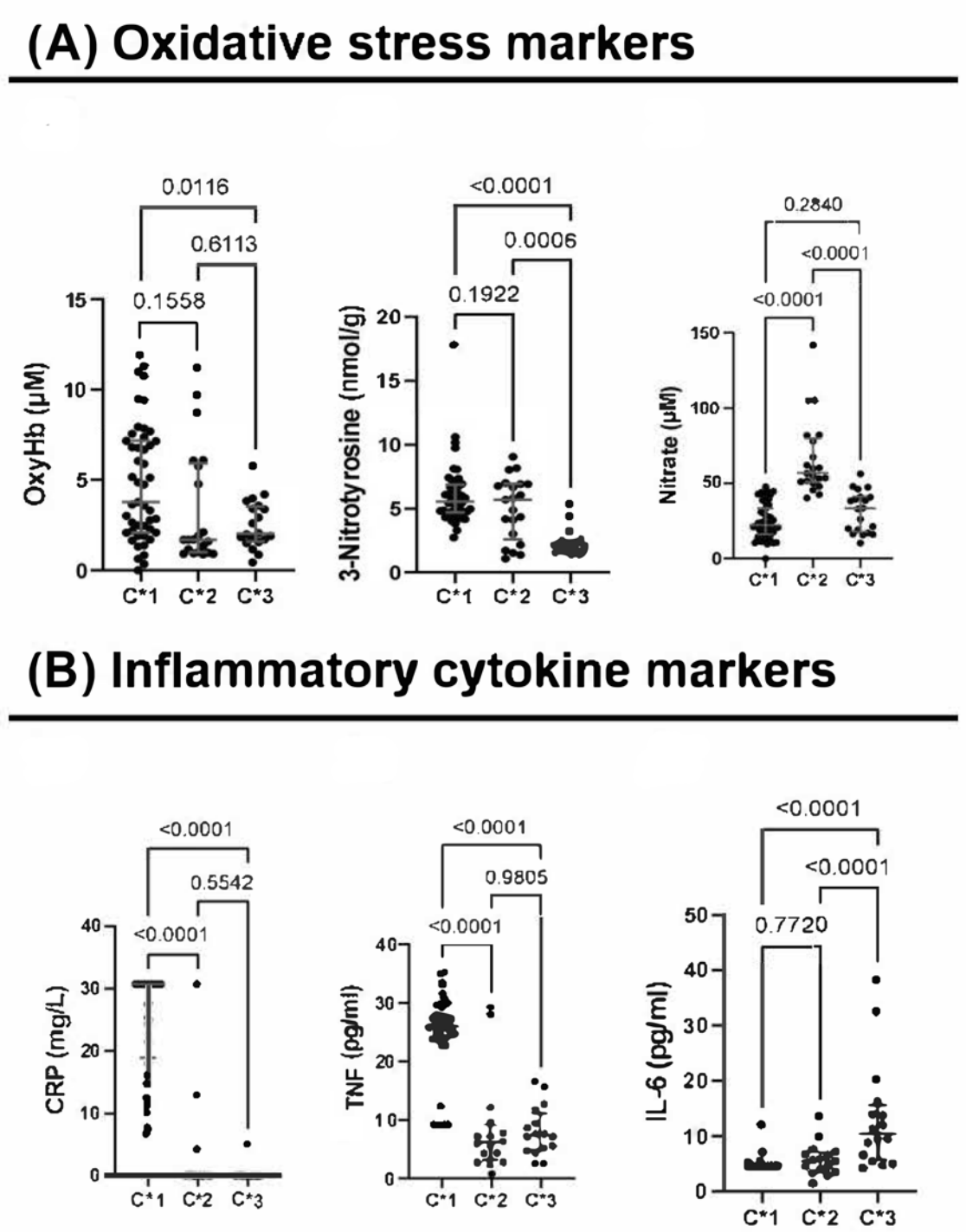

Figure 3. Baseline levels of oxidative and inflammatory cytokine markers with significant differences among the comorbidity-based clusters. (A) Levels of oxidative stress markers and (B) levels of inflammatory cytokines. The marker levels were compared using ANOVA. *P* values above each panel indicate statistical significance between the test groups. C*1: new cluster 1; C*2: new cluster 2; C*3: new cluster 3; CRP: C-reactive protein; IL-6: interleukin 6; oxyHb: oxyhemoglobin; TNF; tumor necrosis factor.

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