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Correlation of Comorbidities and Immune Cellular Response in Biopsies and Surgical Resections of Gout

Syeling Lai[1,2] and Xiaodong Zhou[3]*

[1]Department of Pathology, Baylor College of Medicine, one Baylor Plaza, Houston, TX, 77030, USA
[2]Department of Pathology, Michael E. DeBakey VA Medical Center, 2002 Holcombe Blvd, Houston, TX 77030, USA
[3]Department of Internal Medicine, University of Texas Health Science Center at Houston, 6431 Fannin, Houston, TX, 77030, USA

Abstract

Background: There are many published studies of comorbidities and *in vitro* analysis of inflammatory mechanism in gout, but only few *in vivo* tissue examinations for immune response cells were reported. The association of comorbidities and immune cell response has not been well documented. Our objective is to examine the immune cell types and quantity in gout tissue, identify clinical parameters of gout associated risk factors in corresponding patients, and analyze the association of individual cell type with comorbidities.

Methods: Biopsied or resected tissues from 33 patients diagnosed as gout were used for this study. Cell count was performed on Hematoxylin and Eosin stained sections for macrophages, plasma cells, neutrophils and on immunostained slides for T- and B-lymphocyte.

Results: The mean patient age was 62 year-old with 57.6% patients over 60 year-old. Mean serum uric acid level was 8.5 mg/dl. The average body mass index was 30.6 kg/m^2. Majority of the patients had history of hypertension (93.1%). There were 34.4% and 28.1% of patients admitted history of smoking and alcohol intake, respectively. Hyperlipidemia and diabetes mellitus were seen in 78.8% and 45.5% of patients, respectively. H and E stained sections demonstrated the crystalline deposits rimmed by palisading multinucleated giant cells, macrophages, and chronic inflammatory cells. Average cell counts of T-cells, B-cells, multinucleated giant cells, mononucleated macrophages, plasma cells and neutrophils are: 32.0, 11.8, 7.3, 26.8, 0.38, and 7.4 cells/ High Power Field (HPF) respectively. Of significant values are associations of plasma cells and B-cell with hypertension, T-cell with hyperlipidemia, uric acid with smoking as well as multinucleated giant cells with uric acid.

Conclusion: Most of the gout patients showed an elevated uric acid level. The participating inflammatory cells in local tissues were predominantly T-lymphocytes, macrophages and multinucleated giant cells. Comorbidity factors including hypertension, hyperlipidemia, diabetes, smoking and drinking may precipitate gout by stimulating immune cell proliferation.

Keywords: Gout; Mononucleated macrophages; Multinucleated giant cells; T-cells; B-cells; Uric acid; Comorbidities

Abbreviations: MSU: Monosodium Urate; FFPE: Formalin-Fixed Paraffin-Embedded; H and E: Hematoxylin and Eosin; HPF: High Power Fields

Introduction

Gout is a chronic arthritis resulting from an inflammatory response to Monosodium Urate (MSU) crystals in the tissues. It affects 1% of population with majority being male patients [1-4]. Gout is characterized by elevated serum uric acid level leading to the formation and accumulation of synovial fluid crystals which mediate chronic inflammation. MSU may crystallize when its plasma concentration exceeds its solubility (around 7 mg/dl), which is considered as critical concentration for onset of gout [5,6]. The participating inflammatory cells in the tissues include predominantly T-lymphocytes, some mononucleated macrophages, foreign body multinucleated giant cells and less commonly B-lymphocytes, neutrophils and plasma cells [7]. These together with their cellular products such as variety of chemokines progressively destroy the cartilage and may cause osteolytic irregular destruction of subchondral bone [3,8]. These deposits may extend out from a joint into the soft tissue and cause destruction of the ligaments. This destruction eventually leads to subcutaneous deposits that may erode through the skin. When there is an intense, self-limited bout of acute arthritis with excruciating pain, the patients present as an attack of gout clinically. The hyperuricemia may be caused by diet, increased breakdown associated with malignancy, decreased renal excretion or genetic factors. Reported contributing factors also include hypertension, abnormal lipid metabolism, renal dysfunction, and the use of diuretics [9].

Understanding the inflammatory nature of uric acid crystals is essential to gain insight into the pathogenic mechanism of gout. There are emerging lines of evidence of immunologic responses contributing to the mechanisms and processes involved in MSU-mediated adjuvanticity and inflammation *in vitro* experimental models in the last decades [7,10]. However, only few studies of *in vivo* tissue examination for the cellular characterizations were published [7]. In this study, we examined cell types in gout specimen and searched risk factors in corresponding veteran male patients. To our knowledge, this is the first report of quantitative analysis of immune response cells in tissue specimen in correlation with clinical comorbidities parameters in male veterans. Our purposes of this study are: 1. To examine the immune cell types and quantity in gout tissue; 2. To identify clinical parameters

***Corresponding author:** Xiaodong Zhou, Department of Internal Medicine, University of Texas Health Science Center at Houston, 6431 Fannin, Houston, TX, 77030, USA, Tel: 713-794-7246; Fax: 713-794-7657; E-mail: xiaodong.zhou@uth.tmc.edu

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associated with risk of gout, and 3. To analyze the association of individual cell types with comorbidities.

Materials and Methods

Specimens and clinical characteristics

This study was approved by Baylor College of Medicine institutional review board. Cases were enrolled by snomed code search from pathology report data base and review of electronic chart system. Thirty three patients' specimens previously diagnosed as gout in veteran male patients constitute the materials. All the slides were reviewed to confirm the diagnosis during this study. Specimen sites included upper extremities (13 cases), lower extremities (17 cases) and external ear (3 cases). Most of the specimens were surgically resected tissues (27 cases), the remainders were biopsy samples. Formalin-Fixed, Paraffin-Embedded (FFPE) blocks were made and sections were stained with Hematoxylin and Eosin (H and E) by standard histology protocol. Uric acid levels and body mass index were achieved at or immediately prior to the time specimens were obtained. Hypertension, hyperlipidemia and diabetes were diagnosed by standard clinical guideline. Smoking and drinking history was recorded when the patients continued to have cigarette use and more than occasional alcohol use at the time specimens were obtained.

Immunohistochemistry

Paraffin-embedded blocks were cut on slides and deparaffinized. The sections were stained with CD3 for T-cells and CD20 antibodies for B-cells (Cell Marque, Rocklin, California and Dako, Denmark, California) using Bond Polymer Refine Detection System (Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne NE12 8EW, United Kingdom).

Quantitation of cells

T lymphocyte, B lymphocytes, neutrophils, mononucleated macrophages, multinucleated giant cells and plasma cells were counted on one slide of every specimen. CD3 positive T-cells and CD20 positive B-cells were differentiated by immunostaining. The remainder cell types were identified by morphology based on H and E staining. Dense cellular areas were selected and each category of cells was counted in five high power fields (HPF) at 400X magnification.

Statistical analysis

Association of cell quantitation and clinical data were analyzed by box-and-whisker plot.

Results

All 33 patients were male veterans with the mean age of 62 year-old (ranging from 39 to 84) (Table 1). Mean serum uric acid level at the time specimens were submitted was 8.5 mg/dl (ranging from 5.3 to 11.7 mg/dl). Patients with uric acid levels higher than 7 mg/dl (uric acid becomes insoluble when its plasma concentration is above it) were 87.1%. The mean BMI was 30.6 kg/m^2 (ranging from 22 to 45 kg/m^2). Obese indicated by BMI >30 kg/m^2 was suggested in 48.5%. Majority of the patients had history of hypertension (93.1%). There were 34.4% and 28.1% of patients who admitted history of smoking and alcohol intake, respectively. Hyperlipidemia and diabetes mellitus were seen in 78.8% and 45.5% of patients, respectively.

H and E stained sections of FFPE tissue demonstrated amorphous crystalline deposits rimmed by palisading multinucleated giant cells, macrophages, chronic and occasionally acute inflammatory cells in all cases under transmitted light microscopy (Figures 1A-1C). Polarization of the properly preserved tissues revealed the needle-shaped, double refractile crystals (Figure 1D). Average cell counts of T cell, B cell, multinucleated giant cells, mononucleated macrophages, plasma cells and neutrophils with corresponding ranges were: 32.0 (5.4-121.4), 11.8 (0.2-102), 7.3 (0.2-20.8), 26.8 (3.4-53.2), 0.38 (0-4.2), and 7.4 (0-72.4) cells/HPF, respectively. Eosinophils were occasionally seen in rare

Parameters	Value/PRE	Patients N	Percentage
Age (when specimen was obtained)	<60	14	42.4%
	>60	19	57.6%
Uric Acid Level	<7 mg/dl	4	12.9%
	>7 mg/dl	27	87.1%
	NA	2	
BMI	<30 kg/m^2	17	51.5%
	>30 kg/m^2	16	48.5%
Smoking	P	11	34.4%
	NP	21	65.6%
	NA	1	
Drinking	P	9	28.1%
	NP	23	71.9%
	NA	1	
Hypertension	P	31	93.9%
	NP	2	6.1%
Hyperlipidemia	P	26	78.8%
	NP	7	21.2%
Diabetes	P	15	45.5%
	NP	18	54.5%
Diuretic Use	P	11	33.3%
	NP	22	66.7%

PRE: Presentation, N: Number, NA: Not available
P: Present, NP: Not present, BMI: Body Mass Index

Table 1: Clinical characteristics of patients with gout.

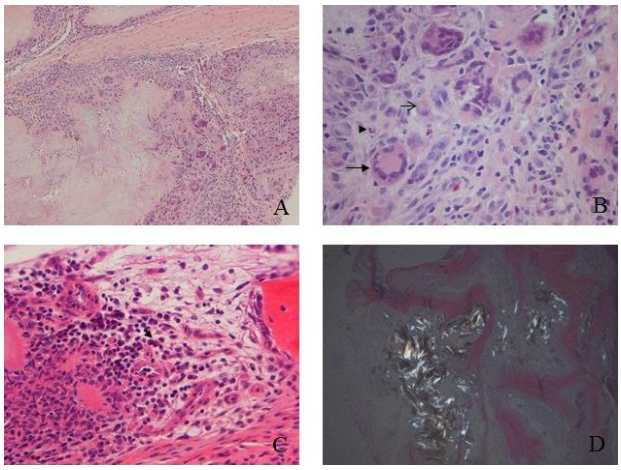

Figure 1: Toe gout involving bone and soft tissue.

A: H and E section shows central crystalline deposits rimmed by multinucleated giant cells and inflammatory cells, and surrounded by soft tissue (100X magnification)
B: Multinucleated giant cells (thick arrow), macrophages (thin arrow) and neutrophils (arrow head) (400X)
C: Amorphous material and inflammatory cells including rare plasma cells (arrow head) in marrow (400X)
D: Polarized light microscopy demonstrates strongly negatively birefringent needle-shaped crystals and surrounding bony plate (40X)

cases. Figures 2A and 2B highlighted CD3 positive T lymphocytes and CD20 positive B lymphocytes by immunohistochemistry.

The correlations of comorbidities of gout and quantitation of immune cell responses were evaluated by statistical significance (Table 2). Of those significant associations, increased plasma cells and B cells were correlated with gout patients with hypertension (p=0.007 and 0.015, respectively). Increased T cells were correlated with hyperlipidemia (p=0.032). Patients who smoked had a higher level of uric acid (p=0.041); while giant cell count in tissues and the level of uric acid demonstrated a negative correlation (p=0.031). The presence of diabetes in gout patients was correlated with the decreased number of macrophages in the tissues with a marginally significant p-value (p=0.052). The results of significant associations were illustrated in the Figure 3 using box plot.

Discussion

Not all patients with increased uric acid develop gout. It is likely that local burden on MSU crystals and general body condition play important roles in gout formation. Acute attacks can be induced by multiple factors such as injuries, cold, food intake, heavy wine drinking and obesity [9]. Most commonly the disease affects middle aged men. In one of the comorbidities studies, Ichikawa et al. found that the increased frequency of hypertension was associated with the duration of gout suggesting that poor control of gout is one of the causes of hypertension [9]. Other studies also showed uric acid predicted hypertension [9,11-13]. In contrast to 28% of gout patients with hypertension reported before Ichikawa et al. [9], our study showed hypertension in 93.9% gout patients. Whether gout can cause hypertension or hypertension predisposes environment or local burden for gout formation is still unclear.

In addition to hypertension, other clinical features of our patients also appeared different from a previous report [9]. The frequency of alcohol use in our patients was 28.1%, hyperlipidemia 78.8%, diabetes 45.5% and mean BMI 30.6 kg/m^2. Compared with the studies by Ichikawa et al. [9] that reported 78%, 56%, 4% and 25 kg/m^2, respectively, the discrepancies could be due to sample size (422 cases in Ichikawa's study) or duration of gout. Patients underwent biopsy or surgical intervention in our study may have longer duration of disease. Our association studies between comorbidities and immune response cells in the tissues indicated that hypertension was associated with increased counts of B-lymphocyte and plasma cells, while hyperlipidemia was correlated with increased T-lymphocyte response. A marginal association (p=0.052) was found between diabetes and the decreased number of macrophages. Another finding of interest in our study, but rarely reported previously is that smoking prevalence was associated with increased uric acid levels, and a lower level of uric acid was associated with an increased number of giant cells in the tissues.

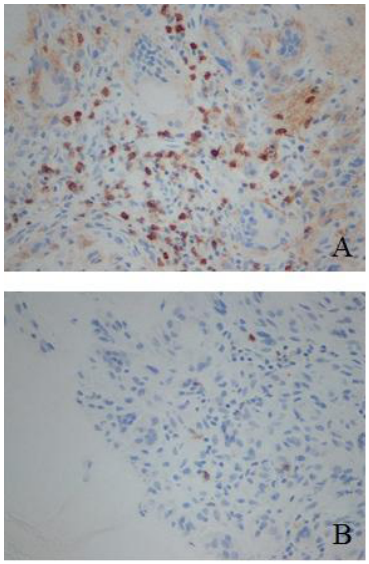

Figure 2: Immunostaining of lymphocytes in toe gout.

A: Diffuse CD3 staining of T-lymphocyte
B: Scattered CD20 staining of B-lymphocyte

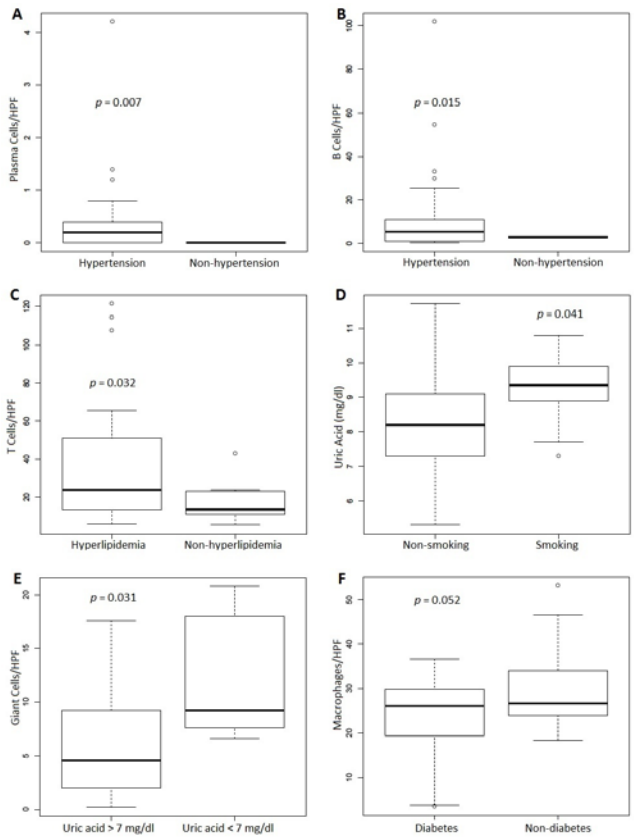

Figure 3: Association between inflammatory cells, uric acid levels and comorbidities.

A: Plasma cells and hypertension
B: B cells and hypertension
C: T cells and hyperlipidemia
D: Uric acid and smoking
E: Giant cells and uric acid
F: Macrophages and diabetes
The line inside the box indicates median value, the box shows 25th and 75th percentiles, the bars indicate 10th and 90th percentiles, and the circles stand for outliers.

Immune response in gout can be triggered by interactions between MSU microcrystals and the local tissue environment [5]. The periarticular joints show a variety of inflammatory cells, such as mononuclear cells and foreign body giant cells in response to deposit crystals [7,14-17]. Several studies examined the characteristics of macrophages as antigen presenting cells within the gouty tophus [7,16,17]. It was reported that the crystal structure may activate innate host defense mechanisms in many ways, and trigger robust spectrum of

			Clinical Features				
	Hyperlipidemia	**Hypertension**	**Uric Acid**	**Diabetes**	**BMI**	**Smoking**	**Drinking**
T-cell	0.032	0.180	0.994	0.483	0.608	0.409	0.061
B-cell	0.678	0.015	0.599	0.339	0.665	0.986	0.113
Giant cell	0.693	0.740	0.031	0.109	0.307	0.490	0.593
Macrophage	0.461	0.732	0.554	0.052	0.661	0.635	0.312
Plasma cells	0.205	0.007	0.714	0.490	0.216	0.337	0.268
Neutrophils	0.340	0.257	0.163	0.141	0.956	0.392	0.402
Uric Acid	0.521	NA	NA	0.795	0.560	0.041	0.333

NA indicates not available or not applicable
Significant correlation=$p<0.05$

Table 2: Correlation of comorbidities of gout and immune cell type by p value.

inflammation including activation of protein receptors, complements, antibodies, chemokines and cytokine response [3]. T cells are the major participating lymphocyte in gout inflammation causing tissue destruction [3,7,8]. It was also reported that uric acid directly promoted human T-cell activation [12].

Although studies of pathogenesis of gout are ample, *in vivo* quantitation tests of inflammatory cells are rarely reported. Our study showed that in gout tissue sections, majority of inflammatory cells were T-lymphocytes, macrophages and foreign body giant cells. B-lymphocytes and plasma cells with scattered neutrophils also presented in the tissues. The paucity of neutrophils within the gout tissues in our study may explain clinical manifestations lack of overt features of inflammatory (tenderness, erythema or heat) in most gout [7,14].

Characterization of comorbidities and immune response cells in gout is valuable for a better patient management, although the mechanisms of association between comorbidities and immune cell responses remain to be identified in future studies.

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