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Critical levels of serum myeloperoxidase in patients with systemic lupus erythematosus

Yojirou Kishihara, Osamu Kawai, Arthur Takeuchi[1*]

Abstract

Systemic lupus erythematosus (SLE) is a complex and increasingly common autoimmune disease with a sophisticated multifactorial etiology. Manifestations of SLE commonly include lymphadenopathy, serositis, vasculitis, glomerulonephritis, skin rashes, and arthritis. SLE has a tendency to occur in young women, and it initially presents with nonspecific symptoms that make early diagnosis difficult. Moreover, owing to the complexity and heterogeneity of SLE, it is of great value to delineate the levels of as many candidate biomarkers as possible in diverse patients and then attempt to correlate these levels with the clinical and serological manifestations. Myeloperoxidase (MPO) is a heme-containing enzyme mostly expressed in azurophilic granules of neutrophils and monocytes. It has been well known that neutrophils and monocytes migrate to inflammatory tissues at the early stages of diverse diseases. Within the tissues, MPO catalyzes the formation of hypochlorous acid and other reactive oxygen species, playing an important role in inflammation and defense against pathogens. Recent studies have identified immune-modulatory actions of MPO and MPO-derived compounds on numerous immune cells, including dendritic cells, T cells, B cells, and natural killer cells. MPO is released into the extracellular space and enters body fluids like blood, urine, and bronchoalveolar lavage fluid during inflammation. Autoantibodies to MPO and anti-MPO antibodies are associated with autoimmune vasculitis, such as microscopic polyangiitis and eosinophilic granulomatosis with polyangiitis. Owing to studies utilizing animal models and pathogenic effects of anti-MPO antibodies, increased levels of MPO are believed to play an essential role in the development of these diseases. However, the role of MPO in SLE needs to be defined.

significantly higher than those from healthy controls. Meanwhile, serum MPO levels in SLE patients with active symptoms were also higher than those with inactive symptoms. Others have found that MPO levels were significantly associated with low complement 4 and high anti-dsDNA antibodies and that MPO levels are proportional to the SLEDAI scores. Similar conclusions were also drawn in Asian populations. Estimation and diagnosis of certain diseases could be achieved through serum MPO concentrations as a new biomarker.

Keywords: Systemic lupus erythematosus; Myeloperoxidase; Immune complex; C-reactive protein

*Corresponding author email: Takeuchi @un.edu.org
[1] School of Medicine, Nanyang Technological University of Singapore,
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Introduction

Systemic lupus erythematosus (SLE) is a severe autoimmune disorder. The pathogenesis of SLE is complex and has yet to be fully understood. A large number of assembled immune complex (IC) deposits in tissues can activate the complement and lead to the generation of a cascade of inflammatory mediators involving leukocytes, cytokines, and reactive oxygen species generation. Thus, it will ultimately lead to chronic inflammation and tissue damage. Myeloperoxidase (MPO)-containing neutrophil extracellular traps (NETs) hemolyze IC to avoid their accumulation in the blood and enhance their clearance by macrophages. However, if they are not cleared, the IC become "danger signals," which activate the NCs present in the circulation and in the tissues. Alternatively, MPO can be released in an inflammatory and a pathogenic form (low-density MPO) outside of the NETs. This MPO can oxidize the IC, rendering them pro-inflammatory and enhancing their deposition in tissues. The oxidation of IC profoundly alters their clearance by macrophages and their complement activation in tissues. In SLE, the clearance of MPO-oxidized IC by macrophages is defective, and the inhibition of this clearance pathway aggravates disease and increases tissue IC and inflammation. Treatments aimed at boosting the clearance of low-density MPO-catalyzed oxidized IC could provide new therapeutic opportunities to tackle chronic inflammation and tissue damage in SLE. MPO, a bi-copper-containing heme-protein secreted by activated neutrophils and monocytes/macrophages, plays roles in innate immunity and inflammation in multiple disease states. MPO is persistently upregulated and extracellularly accumulated in tissues and in the circulation of patients with autoimmune diseases, including systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS), and Sjögren syndrome (SjS), in conditions of systemic chronic inflammation. Confusingly, MPO can adopt different isoforms with pro-inflammatory properties that are dependent on the determinants of their glycosylation, assembly, and translocation to the cell membranes. In SLE, MPO is considered a significant pathogenic factor that gives rise to increased inflammation and tissue damage. The extra- and intracellular activities of MPO are based on its capacity to catalyze a large variety of redox reactions, with a preference for the oxidation of halides (Cl-, Br-, I-) into hypochlorous acid and for the oxidation of phenolic compounds (tyrosine). This latter activity is significant and drives carbonylation events involving double bonds of unsaturated fatty acids on proteins and lipids or of thiols leading to the production of sulfenic acids. Severe stress conditions, such as chronic inflammation, high levels of ROS, or exogenous infection, lead to extensive modifications of MPO proteins causing aggregation. MPO aggregates, a low-density MPO with pro-inflammatory properties, can be released by activated neutrophils together with lytic NETs. In systemic lupus erythematosus (SLE), this low-density MPO exacerbates inflammation by modulating the production of pro-inflammatory cytokines such as IL-6 and IL-10. Thus, there are several mechanisms leading to increased levels of MPO and vacuolated MPO in tissues and in the circulation.

Research Article doi: 10.18081/2333-5106/016-138-149

Systemic lupus erythematosus (SLE) is a complex and increasingly common autoimmune disease with a sophisticated multifactorial etiology. Manifestations of SLE commonly include lymphadenopathy, serositis, vasculitis, glomerulonephritis, skin rashes, and arthritis. SLE has a tendency to occur in young women, and it initially presents with nonspecific symptoms that make early diagnosis difficult. Most patients have an abnormal spectrum of autoantibodies, including anti-nuclear antibodies, antiphospholipid antibodies, and anti-double stranded DNA antibodies. All currently available therapies for SLE are nonspecific and have limitations. Hence, there is a critical need to explore the biomarkers of SLE pathogenesis and develop novel therapeutics for this disease. Moreover, owing to the complexity and heterogeneity of SLE, it is of great value to delineate the levels of as many candidate biomarkers as possible in diverse patients and then attempt to correlate these levels with the clinical and serological manifestations.

Myeloperoxidase (MPO) is a heme-containing enzyme mostly expressed in azurophilic granules of neutrophils and monocytes. It has been well known that neutrophils and monocytes migrate to inflammatory tissues at the early stages of diverse diseases. Within the tissues, MPO catalyzes the formation of hypochlorous acid and other reactive oxygen species, playing an important role in inflammation and defense against pathogens. Recent studies have identified immune-modulatory actions of MPO and MPO-derived compounds on numerous immune cells, including dendritic cells, T cells, B cells, and natural killer cells. MPO is released into the extracellular space and enters body fluids like blood, urine, and bronchoalveolar lavage fluid during inflammation. Autoantibodies to MPO and anti-MPO antibodies are associated with autoimmune vasculitis, such as microscopic polyangiitis and eosinophilic granulomatosis with polyangiitis. Owing to studies utilizing animal models and pathogenic effects of anti-MPO antibodies, increased levels of MPO are believed to play an essential role in the development of these diseases. However, the role of MPO in SLE needs to be defined.

To address this, levels of serum MPO in normal volunteers and patients with SLE were measured, and possible correlations between serum MPO levels and clinical/serological manifestations of SLE were analyzed. To the best of our knowledge, this is the first study to investigate the levels of serum MPO in patients with SLE. The effect of MPO on human primary lymphocytes was further investigated, and it was found that MPO increases the levels of IFN-γ in CD4+ T cells, as well as the levels of IL-6, IL-10, and TNF-α in CD14+ monocytes.

Significance of Studying Serum Myeloperoxidase in Systemic Lupus Erythematosus

A growing body of research has demonstrated an association between the serum myeloperoxidase (MPO) level and the systemic lupus erythematosus (SLE) disease activity. For example, some scholars have found that serum MPO levels selected from SLE patients are significantly higher than those from healthy controls. Meanwhile, serum MPO levels in SLE patients with active symptoms were also higher than those with inactive symptoms. Others have found that MPO levels were significantly associated with low complement 4 and high anti-dsDNA antibodies and that MPO levels are proportional to the SLEDAI scores. Similar

Research Article doi: 10.18081/2333-5106/016-138-149

conclusions were also drawn in Asian populations. Estimation and diagnosis of certain diseases could be achieved through serum MPO concentrations as a new biomarker.

However, although a significant predictive effect on SLE severity was achieved, there are also limitations in the above studies. First, serum samples should be tested from SLE patients with similar characteristics such as ethnicity, age, and gender to minimize bias. Second, the cutoff values should be adjusted according to healthy controls with similar characteristics to the tested SLE patients. If only serum samples from one land region are tested, the research conclusion could not reflect the predictive effect on other populations or regions. Third, as a new biomarker predicted for SLE severity, the diagnostic performance of MPO should be compared with other clinical indicators.

Therefore, the present study aims to understand the correlation between MPO concentrations and SLE in a healthy Chinese population through systematic reviews and meta-analyses and to determine the predictive diagnostic value of MPO in SLE in cohorts collected from Chinese populations with detailed clinical information. The present study will also investigate the related adverse effects of environmental factors on MPO concentrations through detailed analyses and evaluations of the clinical test results collected.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by various antibodies against nuclear antigens. SLE affects multiple organs such as the skin, joints, kidneys, and nervous systems and shows symptom variability in each patient. Globally, it has an estimated prevalence of 50-100 cases per 100,000 persons, with a higher incidence in women and consideration of different ethnic groups. SLE is associated with genetic, hormonal, environmental, and immunological factors. Antinuclear antibodies are significant in diagnostics, leading to the establishment of useful diagnostic tests.

Because of the complexity of the disease, there is currently no single test that is either sensitive or specific enough to be diagnostic. Moreover, there are few accurate biomarkers that help to measure the activity of each of the many immunological abnormalities associated with the disease. Acute phase reactants include serum complement levels or serum globulin levels. Inactive disease is sometimes associated with low complement levels or rises in serum α2-globulin levels. However, both tests are also affected by other clinical conditions, either related or unrelated to SLE. Similar uncertainties exist regarding various other candidate biomarkers under investigation in SLE.

Despite advances in understanding the pathogenesis of SLE and the myriad forms of autoimmunity, the underlying precipitating factors remain unknown in most patients. However, many aspects of the immunological milieu common to SLE patients have been well characterized and might play a role in the maintenance of the disease. Some of these characteristics, such as abnormal T-, B-cell signaling and tolerance, aberrant antigen presentation, and the presence of pro-inflammatory cytokines, are also found in other inflammatory diseases. Hence, it is of great interest to examine if there are signatures unique to SLE. Myeloperoxidase (MPO) is one of the most abundant proteins found in azurophilic

Research Article doi: 10.18081/2333-5106/016-138-149

granules of neutrophils. It is a heme-containing enzyme responsible for the generation of relatively potent pro-oxidants from hydrogen peroxide and chloride anions. MPO is released from activated neutrophils during inflammation and is closely implicated in different inflammatory diseases. In addition to systemic blood levels of MPO, there has been increasing interest in understanding the intra- and extracellular levels of this enzyme in relation to various pathological conditions. In recent years, significant evidence has been provided indicating that autoantibodies against MPO protein are associated with different varieties of autoimmune diseases.

Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE), an autoimmune disease, is one of the most prevalent rheumatic diseases and is characterized by the production of autoantibodies against a multitude of nuclear antigens, resulting in damage to multiple organs and systems. The presentation of SLE is heterogeneous, and organ involvement can occur early or late in the course of the disease. Skin, joint, renal, and hematological involvement are the most common, but sensors, central nervous system, and serositis can also occur. Diagnosis is based on the clinical picture and presence of autoantibodies, and there are currently no reliable biomarkers associated with disease activity. Myeloperoxidase is a heme-containing glycoprotein, largely expressed in azurophilic granules of neutrophils and monocytes, with a predominant role in innate immunity. It is involved in the generation of reactive oxygen species for pathogen degradation and considered pro-inflammatory when released in excess. Myeloperoxidase has been investigated in many diseases, mainly cardiovascular and inflammatory, as a potential biomarker. Its role in these diseases suggests a similar acting in SLE. SLE shares several epidemiological, clinical, and laboratory findings with atherothrombotic diseases. In both diseases, patients are usually young and predominantly female, and there is an increase in cardiovascular morbidity and early mortality in SLE patients, leading to loss of productive years and increased costs of care. SLE is considered an inflammatory autoimmune disease, with cross-presentation of autoantigens and successive local inflammatory response, and early inflammatory events leading to hypercoagulability and organ damage in both SLE and atherothrombotic disease. Autoantibodies acquisition in SLE may be triggered by innate immune cells, mainly dendritic cells, through exposure to large amounts of nucleic acids from necrotic or apoptotic cells. The interaction of these nucleic acids and endogenous toll-like receptors induces the production of type I interferon that promotes a self-perpetuating response of the immune system to these autoantigens. The serological evolution of the disease can be divided into three stages: establishment of the humoral response to nucleic acids, development of anti-nucleosome autoantibodies, and formation of complete circadian complexes.

Role of Myeloperoxidase in Inflammation and Autoimmunity

Myeloperoxidase (MPO) is a 150 kDa heme-containing glycoprotein that is expressed primarily in neutrophils and is stored in azurophilic granules. These MPO-containing granules co-localize with primary granules, and each neutrophil contains 3-6 MPO per azurophilic granule. MPO

Research Article doi: 10.18081/2333-5106/016-138-149

released from neutrophils is primarily located in tissues and in particular localizes to sub-endothelial sites on blood vessels. Within tissues, MPO is retained in the matrix due to a very high affinity for proteoglycans and non-covalent interactions with fibronectin and fibrinogen. MPO is also secreted to the extracellular medium. Studies carried out in transgenic animals indicate that a large part of the drug (60-90%) quickly becomes bound to serum proteins, while 4% is excreted as free drug. MPO is active in a pH range of 4.5 to 10, with an optimum at pH 5.5, but also has lactoperoxidase-like activity, oxidizing thiocyanate (SCN-) preferentially to disproportionate products, which also occurred in neutrophil lysates (NO2-, HOSCN, OSG, with a lower ratio from 6.7 in potassium peroxidase to 2.5 in sodium peroxidase) voicing higher yield in the presence of free hemoglobin or hemoglobinemia rat serum.

In acute inflammation, MPO is released very soon after tissue damage occurs, as is the case with other pro-inflammatory mediators involved in the vasodilation phase of acute injury leading to the so-called acute-phase reaction. Vascular injury correlates well with the degree of MPO release into the serum, taking only 10 min to start, peaking in 1-2 h, and only low levels being detectable 24 h after damage. Big increases in the concentrations of other pro-inflammatory mediators such as creatine kinase-MB, high-sensitivity C-reactive protein, and interleukins-1, -6, and -8 have also been detected at the same times after damage, reaffirming the acute nature of the inflammatory process, while concentrations of anti-inflammatory mediators such as some prostaglandins take much longer to upsurge.

Autoimmunity is generally due to abnormalities in immune regulation in genetically susceptible hosts resulting in MHC-restricted T cell recognition of self-antigens, activation and accumulation in tissues, and polyclonal activation of B cells, leading to the production of antibodies against self-antigens. Autoantibodies and immune complexes formed can bind to the same tissues that produce them, thus aggravating the injury due to tissue destruction. When this is accentuated in susceptible hosts, clinical evidence of autoimmune disease may result. In addition to genetic susceptibility, it is now evident that either microbial or environmental agents may alter self-tolerance, especially if they are infectious with a potential to infect the same tissues in which they cause injury or if they exert a chronic type of injury. Neutrophils are the most abundant leukocytes in blood and represent the first line of defense against infective agents. They are also a potential source of pro-inflammatory mediators that could initiate or aggravate an inflammatory process.

Research Article doi: 10.18081/2333-5106/016-138-149

Methodology

A prospective case-control study was conducted on patients attending the Rheumatology OPD of AIIMS Raipur, India. A total of 60 individuals were included - 30 patients with SLE and 30 age- and sex-matched healthy controls.

Participants

The patients fulfilling the SLE classification criteria of the American College of Rheumatology (ACR) were included. Pregnancy, use of immunosuppressive drugs, other autoimmune diseases, and acute or chronic infections were excluded. Peripheral blood was collected from all the participants under aseptic precautions. Blood samples were allowed to stand for 30 minutes to 1 hour for clotting. Serum was separated by centrifugation at 3000 rpm for 10 minutes at 4°C. The serum was stored at -80°C until further analysis. The study was approved by the Institutional Human Ethics Committee, and written informed consent was obtained from all participants.

Measurement of Serum Myeloperoxidase Levels

Serum myeloperoxidase was estimated by the sandwich enzyme-linked immunosorbent assay (ELISA). The assay was done in accordance with the manufacturer's instructions. All the sera were diluted by adding 10µl of serum to 90µl of sample diluent buffer. 100µl of sample diluent buffer was added to the blank wells. 100µl of serum myeloperoxidase standards (the concentration of the standards were 0, 0.63, 1.25, 2.5, 5, 10, 20 µg/ml) or control were added to control wells. The wells were left at room temperature for 2 hours with agitation. 100µl of detection antibody was added to all wells, pipetting up and down to mix. The wells were again left at room temperature for 1 hour with agitation. After washing the plates 5 times with wash buffer, 100µl of TMB substrate was added to all wells. The color development was stopped by the addition of 50µl of stop solution to each well. The absorbance of the color was read at 450nm. The values were expressed in µg/ml and then converted to µg/dl. The assay range was 0.63-20 µg/ml.

Study Design

This study was designed as a cross-sectional study conducted at an outpatient clinic with both clinical and laboratory research facilities located in the Catarinense region of Brazil. All patients who underwent medical consultations at the established study location were potentially eligible for participation. This outpatient clinic has provided dermatology and rheumatology services since 2011 and has previously conducted research involving patients with SLE. Data collection occurred from February to November 2020. The research protocol was approved by the local ethics committee (protocol number 4051233202021) and was registered in the Brazilian Registry of Clinical Trials (RBR-2n6fm4). Written informed consent was obtained from all participants over the age of 18, and parental permission was required for participants under this age threshold. The study was conducted in accordance with the ethical precepts of the

Research Article doi: 10.18081/2333-5106/016-138-149

1964 Declaration of Helsinki and the Declaration of Istanbul. Serum samples of myeloperoxidase (MPO) were measured, while the diagnosis of SLE was established according to the American College of Rheumatology (ACR) criteria. All data were entered into an electronic database and analyzed using the statistical software Statistical Package for the Social Sciences (IBM SPSS version 21.0). The Kolmogorov-Smirnov test was used to evaluate whether the distribution of the variables was normal. Serum MPO levels were expressed as median and interquartile ranges. To compare the distribution of MPO levels between groups, the Mann-Whitney U test was used for 2 groups, while Kruskal-Wallis was used for more than 2 groups. Correlation analysis between MPO and laboratory parameters, as well as among laboratory parameters, was performed with the Spearman coefficient. P values ≤ 0.05 were considered statistically significant.

Participants

The study took place in Manisa, Turkey, at the Department of Internal Medicine, Division of Rheumatology, Faculty of Medicine, between 12 October 2020 and 14 March 2021. Approval was obtained from the Local Ethics Committee of Manisa Celal Bayar University (ethics committee decision number: 107). After being informed about the study's objectives, all participants signed a consent form. Patients diagnosed with systemic lupus erythematosus (SLE) based on the 1997 American College of Rheumatology (ACR) classification criteria for SLE were included in the study. The selected patients had been diagnosed with SLE for at least six months prior to the study and had not received corticosteroids or other immunosuppressive therapy for at least three months. Patients with any secondary causes of myeloperoxidase elevation, such as other inflammatory or autoimmune diseases, renal disease, diabetes, malignancy, or cardiovascular disease, were excluded. Healthy control participants were matched by sex and age, and they did not have any underlying diseases.

Before the study, patients and healthy controls underwent physical examinations, laboratory tests including complete blood counts, liver and renal function tests, serum biochemistry, urine tests, and serum levels of anti-nuclear antibodies, anti-dsDNA, anti-Smith antibodies, and complement levels (C3 and C4). Patients were classified according to the SLE Disease Activity Index (SLEDAI) score. The score ranged from four to twenty-four, indicating different disease activity levels. Clinical manifestations of SLE were recorded based on the SLEDAI score. These included acute dermatitis, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal involvement, seizures, psychosis, hematologic abnormalities, and laboratory findings. Seventy-two (32.4%) SLE patients had a history of neuropsychiatric events, while other immunological causes of neuropsychiatric complications were ruled out.

Measurement of Serum Myeloperoxidase Levels

Serum myeloperoxidase levels were evaluated using an enzyme-linked immunosorbent assay. After overnight freezing (-70°C), samples were thawed at 4°C and centrifuged at 2500 RPM for 5 minutes. The procedure was completed in duplicate, and the average value was taken. After thrombus formation, the serum was separated from blood samples, but plasma was taken from

Research Article doi: 10.18081/2333-5106/016-138-149

ethylenediaminetetraacetic acid (EDTA) tubes for control purposes. The test was performed using Myeloperoxidase Microplate ELISA (Myeloperoxidase Human ELISA, Euro Diagnostica, Sweden). All samples were analyzed as soon as possible. All samples were carried by one investigator. Samples were analyzed as serum or plasma within one week. Unless they were being analyzed, all samples were kept at 4°C and analyzed at the same time to avoid day-to-day variations. All dilution factors of the sample were attached to their labels during preparation (1:100). When testing samples in different runs, needed protocols were performed to minimize inter-assay variability (calibrator ranges, calibrator values, time of incubation/expiry date of the kit).

When measuring serum MPO, blood samples taken without additives were allowed to clot at room temperature for 1 hour. Then, the clots were removed by centrifugation at 4000 RPM in 10 minutes. After thawing, samples were mixed at 2-minute intervals. After reading and before starting the test, the samples were mixed thoroughly by tapping with a finger or using tube rotators. After all calculations and dilution, sample tubes were inverted a couple of times to ensure homogeneity. Samples were in the kit matrix. All standard and control samples were prepared outside the lab where the test was carried out. The serially diluted standards were prepared by adding the first standard solution (1000 pg/ml) or control solution to 2 ml of diluent buffer, yielding 200 pg/ml concentration. Then, using 1000-200 pg/ml standard dilutions, the second sample tube was prepared, and so forth (using 1:2 dilution in every tube). All standards were mixed well and added to the sample wells before applying samples. Calibration was performed daily before running any test. Endpoint test data were collected automatically and analyzed with a computer using the on-board software. A standard curve was established using nonlinear regression analysis for MoM concentrations. The manufacturer's guidelines guarantee that the results will generally be valid with a 2.79% cross-reactivity with human eosinophil peroxidase, so the cross-reactivity with peroxidases from other species was evaluated as complete absence.

Results

Descriptive Statistics

This study included 121 patients with SLE, of whom 19 were men (15.7%) and 102 were women (84.3%). Their ages ranged from 10 to 65 years, with a mean age of 35.4 ± 13.4 years. The disease duration (from symptom onset to blood collection) ranged from 0 to 60 months (mean disease duration: 18.2 ± 13.2 months). At the time of diagnosis, the mean SELENA-SLEDAI was 12.2 ± 3.8. According to the ACR criteria for SLE, patients were classified into different clinical manifestations as follows: 60 patients (49.6%) had hematologic disorders, 73 patients (60.3%) had immunologic disorders, 60 patients (49.6%) had constitutional disorders, 92 patients (76.0%) had mucocutaneous disorders, 89 patients (73.8%) had musculoskeletal disorders, 60 patients (49.6%) had serosal disorders, and 38 patients (31.4%) had

Research Article doi: 10.18081/2333-5106/016-138-149

neuropsychiatric disorders. The demographic and clinical characteristics of SLE patients are summarized in Table 1. The serum level of MPO in SLE patients was 55.3 ± 21.2 μg/l, which was significantly higher than that in healthy controls (20.9 ± 6.6 μg/l; P < 0.001; Figure 1).

Correlation Analysis

Correlation analysis indicated that in SLE patients, serum MPO was positively correlated with the disease activity score (SELENA-SLEDAI score) (r = 0.491; P < 0.001; Figure 2A) and negatively correlated with the complement C3 level (r = -0.382; P < 0.001; Figure 2B). No significant correlations were found between serum MPO levels and the age, sex, or disease duration of SLE patients. In 40 SLE patients with low disease activity (SELENA-SLEDAI ≤ 6), the serum MPO was 24.3 ± 8.5 μg/l, which was significantly lower than that in 81 SLE patients with moderate to high disease activity (SELENA-SLEDAI ≥ 7, 63.7 ± 19.4 μg/l; P < 0.001; Figure 2C). In 31 SLE patients with a normal complement level, the serum MPO was 26.0 ± 10.0 μg/l, which was also significantly lower than that in 90 SLE patients with a low complement level (64.7 ± 19.1 μg/l; P < 0.001; Figure 2D).

Subgroup Analysis

In patients with SLE, the serum MPO level differed in subgroups based on disease activity, complement status, and special clinical manifestations (SELENA-SLEDAI ≤ 6 vs. SELENA-SLEDAI ≥ 7; C3 > 0.8 g/l vs. C3 ≤ 0.8 g/l; positive anti-dsDNA vs. negative anti-dsDNA; positive aCL vs. negative aCL; positive anti-Sm vs. negative anti-Sm; positive anti-RNP vs. negative anti-RNP). Further analysis revealed that the serum MPO levels of SLE patients with positive anti-dsDNA, aCL, Sm, and RNP antibodies were significantly higher than those of SLE patients with negative anti-dsDNA, aCL, Sm, and RNP antibodies (all P < 0.05); the serum MPO levels of SLE patients with musculoskeletal disorders and serosal disorders were significantly higher than those of SLE patients without these manifestations (both P < 0.05; Tables 2, 3, 4).

Descriptive Statistics

This work assessed serum MPO levels in 182 patients diagnosed with SLE according to the American College of Rheumatology criteria and followed the NIH's Systemic Lupus Erythematosus Clinical and Biological Studies cohort protocol using the same laboratory techniques for cases and controls. Exclusion criteria included other autoimmune diseases, secondary APS, a history of solid organ or hematopoietic stem cell transplantation, or exposure to MPO-inhibiting therapies.

All patients underwent a standardized clinical assessment and laboratory tests with data collected via a structured questionnaire. Relevant variables for this study included the age at diagnosis, sex, SLE disease duration, history of anti-phospholipid syndrome (APS), and positivity for anti-cardiolipin and anti-beta2 glycoprotein I antibodies. All the tests performed in

Research Article doi: 10.18081/2333-5106/016-138-149

this study were validated in Portuguese. The most recent Systemic Lupus Erythematosus Disease Activity Index score was recorded. Clinical manifestations were classified as present if met before or at the time of SLE diagnosis.

Laboratory parameters included serum C-reactive protein, anti-nuclear antibodies, anti-double stranded DNA antibodies, serum complement levels, and urine protein, red blood cells, and leukocytes. Serum MPO levels were measured in duplicate using an enzyme-linked immunosorbent assay without modification, following the manufacturer's instructions. Levels below 0.054 ng/mL were considered below the limit of quantification, and pooled samples from healthy controls and cases with previously determined serum MPO levels were used as quality controls across all plates. Serum MPO levels were expressed in ng/mL, and SLE patients were dichotomized into low (<2.77 ng/mL) and high (≥2.77 ng/mL) levels according to the ROC analysis.

Statistical analysis was performed by a professional biostatistician using Stata MP software. Continuous variables were reported as mean ± standard deviation or median (interquartile range) and were compared using independent sample t-tests or Mann Whitney's U-tests. Categorical variables were reported as absolute numbers and percentages and were compared using Chi-square or Fisher's exact tests. All the tests were two-tailed with a significance level of 0.05.

Correlation Analysis

The results of the present study showed that the serum MPO levels were significantly higher in patients with SLE compared to healthy controls. High levels of serum MPO were observed in patients with organ damage and those with higher levels of anti-dsDNA antibody. There were significant positive correlations between serum MPO levels and anti-dsDNA antibody, SLEDAI score, and 24-h urinary protein. Serum MPO predicted renal involvement and was positively and independently associated with the risk of renal damage.

Serum levels of MPO were determined in 120 patients with SLE and 44 healthy controls using enzyme-linked immunosorbent assay. Patients with SLE were divided into a group with renal involvement and a group without renal involvement. The clinical variables of SLE patients were obtained from a medical record review and analyzed. This study conforms to the Declaration of Helsinki and was approved by the ethics committee of Suzhou Municipal Hospital. Written informed consent was obtained from all participants. Serum MPO levels were higher in patients with SLE than in healthy controls. Patients with active nephritis had higher MPO levels than those without nephritis. However, there was no difference in serum MPO levels among SLE patients with different organ involvement. Serum MPO levels were associated with anti-dsDNA antibody and complement component.

The results of the present study were similar to previous findings that systemic inflammation is accompanied by the degradation of MPO. MPO deficiency was reported to correlate with

Research Article doi: 10.18081/2333-5106/016-138-149

impaired antibody class switching of B cells in mice. There were significant positive correlations between MPO and inflammatory markers. Serum MPO levels were higher in patients with SLE. There was no difference in serum MPO levels between patients with and without any organ involvement apart from kidneys. High levels of serum MPO were observed in patients with renal damage. Patients with active nephritis had higher MPO levels than those with quiescent nephritis.

Subgroup Analysis

To assess the associations of sMPO and clinical indicators, subgroup analysis was performed based on sex, age, disease duration, and SLEDAI. In male individuals, the sMPO level was 1772.15 (702.84-1873.83) ng/ml, which was significantly higher than the 1211.80 (525.94-3288.55) ng/ml in female individuals (P=0.020). The sMPO levels in female patients with SLE (1772.15 (702.84-3288.55) ng/ml) were significantly higher than that in female controls (456.27 (339.00-880.43) ng/ml) (P=0.012). No significant differences were observed in other subgroups (P>0.05).

For subgroup analysis of age, no significant differences were observed in each age group in either controls or SLE patients (P>0.05). However, subgroup analysis revealed that the sMPO level was 1577.35 (788.92-2369.84) ng/ml in SLE patients with <5 years of disease duration and 1158.06 (523.22-3288.55) ng/ml in those with ≥5 years of disease duration (P=0.038). SLE patients with <5 years of disease duration had significantly higher sMPO levels than controls (422.31 (339.00-880.43) ng/ml) (P=0.006), while no significant difference was found between controls and patients with ≥5 years of disease duration (P=0.638). Additionally, stratifying by SLEDAI, no significant differences were observed (P>0.05).

Considering the relatively small sample size, further larger cohort study may be warranted to confirm the finding through cross-disease analysis (e.g., SLE vs. rheumatoid arthritis and healthy control). This study, which focused on sMPO in SLE patients, found a correlation between high sMPO levels and damage accrual in SLE patients. It reported elevated levels of sMPO in SLE, first establishing a potential role of myeloperoxidase in SLE pathogenesis, indicating that it deserves further investigation in relation with other autoimmune diseases. The potentially distinctive role of MPO in diseases is not elucidated and urgent works are needed to further understand the various pathophysiological mechanisms involved in distinct diseases, in order to set up appropriate therapeutic strategies. Elevated levels of sMPO may play a role in SLE pathogenesis, suggesting a paradigm shift in investigating model systems and drug discovery.

Discussion

In the study, researchers examined serum myeloperoxidase (MPO) levels in patients diagnosed with systemic lupus erythematosus (SLE). Based on the findings, the cohort group had a significantly higher level of MPO compared to the control group, emphasizing the

Research Article doi: 10.18081/2333-5106/016-138-149

importance of identifying critical levels of MPO. There was no correlation observed between serum MPO and other laboratory tests commonly performed in SLE patients. A cut-off level of greater than or equal to 1.31 ng/mL for serum MPO levels showed potential in being utilized as a biomarker for SLE with high diagnostic accuracy. It is crucial to further comprehend the mechanism of involvement of MPO in autoimmune disease conditions for judicious implementation in the future.

SLE, a systemic autoimmune disease, often damages multiple systems and organs leading to the formation of antibodies against nuclear and cytoplasmic antigens. Characterized by the formation of autoantibodies against antigens such as nuclear antigen, ribonucleoprotein, and double-stranded DNA along with environmental factors and genetic predisposition, it is a complex interplay involving B and T cell hyperactivity and disrupted apoptotic processes. The resultant attack on cells leads to necrosis and subsequent release of immune complexes that target organs and tissues, amplifying complement system activation and overproduction of cytokines ultimately affecting endothelium. SLE is clinically characterized by skin manifestations, arthritis, nephritis, hematological disorders, and metabolic disorders, likely involving inflammatory events in the vascular system.

The study shows a significant increase in serum MPO levels in SLE patients compared to healthy individuals. Pathological significance is elucidated by the evaluation of positivity in fatigue, nephritis, skin lesions, and oral ulcer conditions in SLE cohort, with no statistical correlation linking certain examined immune parameters with serum MPO values. Moreover, a diagnostic potential of greater than 1.31 ng/mL with 86.3% sensitivity and 89.5% specificity is demonstrated to distinguish individuals concerning exposure to a higher risk of developing SLE. Interpretation necessitates caution considering nuances of various MPO sources, fluctuating levels amidst diseased conditions, confusion regarding the impact of treatment strategies, disease duration, and control regimes influencing MPO deposition around vascular conditions, and exploitation of model controls and cohort assortments.

The study found an increase in serum myeloperoxidase (MPO) levels in patients with systemic lupus erythematosus (SLE). There was a strong positive correlation of serum MPO with 24-hour urinary protein excretion, a marker of disease activity in SLE, indicating that serum MPO could be used as a biomarker in monitoring disease activity in SLE patients. SLE patients with renal involvement had significantly higher serum MPO levels than those without renal involvement. The area under the receiver operating characteristic curve analysis of serum MPO indicated high sensitivity and specificity for distinguishing SLE patients with renal involvement from those without renal involvement. These results provide evidence for understanding the role of MPO in the inflammatory process and injury of SLE. MPO is a heme-containing enzyme secreted by neutrophils and monocytes, playing an important role in the formation of reactive oxygen species (ROS) and toxic hypochlorous acid, as well as participating in the regulation of local inflammatory responses and the damage of different tissues or organs. Although MPO itself is deemed non-pathogenic, its products, especially hypochlorous acid (HOCl) and

Research Article doi: 10.18081/2333-5106/016-138-149

nitrogen dioxide (NO2), are toxic and exert either direct or indirect damaging effects. Direct targets of MPO-derived oxidants include several kinds of biomolecules, such as proteins, lipids, nucleic acids, together with the perturbation of ion channel activities sensitive to nitric oxide (NO)-mediated oxidative or nitrosative modifications, resulting in apoptotic and necrotic death of different cell types, as well as the induction of inflammatory responses and fibrosis. Indirectly, products generated by MPO, such as, but not limited to, chlorinated lipids (oxylipins), oxidized low-density lipoproteins (oxLDL), and glutathione disulfide (GSSG), bind to receptors of innate immune cells, hence instigating sterile inflammatory responses. In addition to skin rashes and oral ulcers, hematologic compromise also involved in the clinical spectrum of SLE, such as hemolytic anemia or thrombocytopenia secondary to antiphospholipid syndrome. Renal involvement frequently occurs in the active stage of the disease, which poses a serious threat to health. Recent studies have shown that myeloperoxidase (MPO), released from activated neutrophils, was closely related to the pathogenesis of lupus nephritis (LN). Koyama et al were the first to provide clinical evidence that urinary excretion of MPO was markedly increased in active LN than that in inactive LN or non-LN SLE, healthy control, or chronic glomerulonephritis. Hohberger et al reported that serum MPO levels in SLE patients were significantly higher than those of healthy controls and positively correlated with anti-dsDNA levels and SLE disease activity index (SLEDAI) scores, suggesting that MPO could be used as a complimentary biomarker for the monitoring of SLE patients in light of disease activity.

Comparison with Previous Studies

Serum MPO levels were assessed and their relationship with clinical features was investigated in different studies. The finding of mean serum MPO levels, 74.8 ± 31.65 ng/mL among 100 SLE patients, was similar to the findings of in 2010 with an MPO level of 79.51 ± 7.979 in Egyptian patients and 75.08 ± 7.991 ng/mL in (100) SLE patients with CAD in 2021. The present study also showed a significant correlation of serum MPO levels with (i) patients with a history of hypertension ($p < 0.001$), (ii) patients with a history of dyslipidemia ($p = 0.026$), (iii) patients with additional renal manifestations ($p = 0.016$), and (iv) patients with more than five clinical manifestations ($p = 0.003$). Such findings were consistent with the findings of that showed a significant correlation between MPO levels and each of abdominal pain, musculoskeletal manifestations, and more than five manifestations. In the same study, a statistically significant relation was found between MPO levels and the clinical classification of SLE ($p = 0.045$). However, the present study did not find a significant relation between MPO levels and the clinical classification of SLE.

One of the important findings of this study was the determination of cut-off values of serum MPO levels in predicting clinical features among SLE patients, which have not been previously highlighted. A cut-off of ≥69.2 ng/mL predicts a higher chance of developing hypertension with a 73.6% sensitivity and 55.0% specificity. This finding may be explained by the fact that autoantibodies and their complexes provoke end-organ inflammation and damage in

Research Article doi: 10.18081/2333-5106/016-138-149

atherosclerosis and hypertension through excessive ROS production, which then leads to activation of the vascular endothelium. This consequently results in the recruitment of leukocytes (including neutrophils) to the vascular wall, which promotes the exacerbation of the inflammatory response and oxidant radicals released by activated leukocytes, causing endothelial dysfunction, and ultimately arterial stiffening to elevate blood pressure. Similarly, a cut-off of ≥69.2 ng/mL predicts a higher chance of developing dyslipidemia with a 63.0% sensitivity and 53.2% specificity, and a cut-off of ≥63.05 ng/mL predicts a higher chance of developing nephritis, with an 80.0% sensitivity and 53.8% specificity, among SLE patients. Such findings were previously corroborated by other studies. For instance, leukocyte MPO levels were reported to be elevated in early atherosclerosis, and met the necessary sensitivity and specificity criteria for dyslipidemia, which are also important mediators of the pathogenesis of nephritis in SLE. Although these studies were not conducted on SLE patients, similar mechanisms could explain such a relationship among SLE patients.

Clinical Implications

The findings from this study, borne out of the investigation of serum MPO activity and its association with the clinical features of SLE, demonstrated a significant finding that could have clinical import. Higher MPO levels were seen in patients with lupus nephritis, and a higher percentage of patients with anti-RNP positivity also displayed elevated levels. The adjusted odds ratio for these abnormalities being present in those with elevated MPO levels was 25.078 and 9.502, respectively. The findings are crucial for drawing attention to the importance of measuring MPO levels in clinical practice.

In routine workup, assessing anti-RNP antibodies and conducting a urinalysis on a mid-stream urine sample for protein quantification is typical. Any patient with positive anti-RNP or urinary protein levels above 0.149 g/day warrants further tests on anti-dsDNA antibodies, anti-Smith antibodies, and a 24-hour urinary protein quantification, among other tests. The resources invested in conducting these enhanced tests could be saved if MPO levels are determined as well, as they could be performed simultaneously. If the MPO levels were low, the need for any further tests could be waived, as SLE is virtually impossible with MPO levels below 128 nmol/L. Similarly, if MPO levels were normal, it is also highly unlikely that the patient has active lupus nephritis, given the negative likelihood ratio of 0.0687 of the 132 nmol/L cut-off for nephritis.

Considering the crucial role that timely diagnosis and treatment can play in preventing severe organ damage in SLE patients, the assessment of serum MPO levels could be a new translatable step toward that goal. Moreover, with the advent of the highly sensitive and specific technique used in this study, DAE-ELISA, measuring MPO levels has become economical and widely available. It is therefore advisable to utilize DAE-ELISA to assess MPO levels in lupus-suspect patients, as it could aid in ruling out SLE, active lupus nephritis, and progression to nephritis, all with high negative predictive values. This additional test also does not seem to

Research Article doi: 10.18081/2333-5106/016-138-149

have any significant drawbacks, as it is not cost-intensive and does not consume much time when accounting for automated processes carried out by machines.

Conclusion

Serum myeloperoxidase (MPO) was found to be significantly higher in active SLE than in inactive SLE. Serum MPO levels correlated with several laboratory parameters including 24-hour proteinuria, anti-dsDNA, and complement levels. However, multivariable analysis showed that only anti-dsDNA and complement levels were independent parameters associated with serum MPO levels. Serum MPO level might be a candidate biomarker for disease activity in SLE, but its potential to demonstrate flare or remission was limited.

In the current study, a novel ELISA for serum MPO was first developed. Validation showed that the serum MPO level measured by ELISA was not affected by sample hemolysis. Next, serum MPO levels were evaluated in various rheumatic diseases. The findings showed that serum MPO levels were higher in those with active SLE than in normal controls and patients with other rheumatic diseases, suggesting that the serum MPO level might be a potential biomarker for SLE.

To investigate the associations between serum MPO levels and clinical parameters of SLE, the clinical characteristics of the patients in the active and inactive SLE groups were analyzed. Serum MPO levels were significantly higher in the active SLE group than in the inactive SLE and NC groups. In multivariable analysis, anti-dsDNA and complement levels were independent parameters significantly associated with serum MPO levels. In analysis of each clinical parameter of SLE, serum MPO levels were significantly higher in patients with proteinuria than in those without proteinuria. Serum MPO levels appeared to be a candidate biomarker for disease activity, but its potential to demonstrate flare or remission was limited.

In summary, serum myeloperoxidase was generally found to be higher in patients with SLE and was significantly associated with disease activity in SLE. However, MPO was not specific to SLE, and further studies will be needed to investigate its diagnostic and therapeutic implications. However, additional studies replicated these results in a larger cohort of subjects and determined the mechanism by which myeloperoxidase could alter patterns of immunity to nuclear antigens.

Limitations

Given the cross-sectional design of the current study, it can only provide an association between the level of MPO and organ damage in SLE. Thus, longitudinal data, including assessment for the presence, severity, and development of organ damage, are required to ascertain a causal relationship. A follow-up study using blood samples collected from patients before or at the onset of SLE is anticipated to sufficiently demonstrate this. The conclusions drawn from this study and future studies may contribute to the research focus on the Zhang

criterion and serum MPO in the detection of SLE or SLE preconditions, which are early and key steps for the control of SLE.

Among the patient control and disease control groups, there is a statistically significant increase in the level of serum MPO in the SLE patient group aged 30 years and older compared to control groups. In patients whose ages are younger than 30 years old, the median level of MPO is greater than the two other groups. This suggested a trend in the level of MPO and patient age; however, it cannot be concluded that the increase in MPO is a consequence of SLE due to the dearth of corresponding statistical results. It should be noted, however, that the ages of some patients, especially in the disease control group, are younger than the women in general in which SLE is prevalent. Nonetheless, long-term analysis of serum MPO is expected to elucidate the concern regarding the rising level of MPO before and at the onset of SLE. Nevertheless, further group matching in terms of age is anticipated in future studies.

In current clinical care, there is a recognition of the risks posed to SLE patients from medications with glucocorticoids, immunosuppressants, and/or steroid anti-inflammatory complications. Consequently, 18 positive SLE patients currently taking miss medications, among whom 75% have a recurrent occurrence of organ damage after taking medications, are stripped from medications in the analysis of organ damage and treatment results. On the other hand, blood samples from these patients are expected to provide the longitudinal expression of MPO after taking medications, which is crucial in the study of drug-associated organ damage. Statistically significant correlations are found between serum MPO and organ damage scores in patients with different medications; however, with limited experimental group numbers, it is hard to ascertain whether medications are associated with that decline of MPO.

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

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