Peripheral Classic and Intermediate Monocyte Subsets as Immune Biomarkers of Systemic Lupus Erythematosus Disease Activity

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ABSTRACT

***Background**: Monocytes are evolutionarily preserved innate immune cells that play essential roles in immune response regulation. Three activated monocyte subsets—classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and nonclassical ($CD14^{+}CD16^{++}$)—are associated with systemic lupus erythematosus (SLE) progression. This study aims to determine the association of monocyte subsets with SLE disease activity.* ***Methods**: A cross-sectional study involving 25 patients with SLE was conducted. Blood samples were collected, and monocyte subsets were identified using flow cytometry. Patients were grouped by disease activity using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) into inactive (SLEDAI-2K ≤ 4) and active (SLEDAI-2K > 4). The cutoff for monocyte subsets was determined using Receiver Operating Characteristic (ROC) analysis.* ***Results**: Nine active and 16 inactive subjects were identified. Compared with individuals without active disease, individuals with active disease had significantly lower mean classical monocyte subsets (71.9% vs 88%, p = 0.008), and higher median intermediate monocytes (29.1% vs 11.1%, p = 0.019). The median nonclassical monocyte subsets were not significantly different between the two groups. The cutoff for classical monocytes in active disease was ≤72.2%, AUC = 0.788, p = 0.021, with 66.7% sensitivity and 87.5% specificity; for intermediate monocytes, it was >22.3%, AUC = 0.788, p = 0.014, with 66.7% sensitivity and 100% specificity.* ***Conclusion**: Classical and intermediate monocytes could be considered as immune cellular markers for identifying active SLE.*

***Keywords:** Monocyte subset, SLE, Classical monocytes, Intermediate monocytes, Disease activity.*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease affecting multiple organ systems, such as the skin, joints, and kidneys.[1,2] Disease activity in SLE in clinical practice is assessed using a scoring system known as the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), which comprises several clinical findings and laboratory markers.[2]

Despite the established markers of SLE activity, the search for new markers continues.[2] The role of hematologic parameters as markers of SLE activity has become a topic of increasing interest in recent years. One recent finding regarding this topic is the ratio of neutrophils, basophils, eosinophils, monocytes, and platelets to lymphocytes, which has been found to indirectly reflect subclinical inflammation.[2]

Monocytes are innate immune cells that are essential in protecting against pathogens while producing several inflammatory cytokines. Changes in monocyte activities can affect host defense and cause the development of inflammatory diseases.[3] Recent studies categorize monocytes into three subsets according to CD14 [lipopolysaccharide co-receptor] and CD16 (FcγRIII) expression levels: $CD14^{++}CD16^{-}$, $CD14^{++}CD16^{+}$, and $CD14^{low}CD16^{++}$ are referred to as classical, intermediate, and nonclassical monocytes, respectively.[3,4]

Immunological changes in many autoimmune diseases involve autoantibodies and autoreactive lymphocytes, which indicates that the adaptive immune response is important in pathogenesis. These changes, however, cannot be the only changes involved in the development of autoimmune diseases because crosstalk with the innate immune system is needed for activation in the inflammatory response against pathogens. Therefore, the innate immune response may play a necessary and irreplaceable role as well.[5] Investigation of activated monocytes in autoimmune diseases could bridge the knowledge gap in immune response regulation.

Evidence suggested that monocyte activation was associated with the progression of autoimmune diseases, such as SLE. Hence, we aimed to determine the association of monocyte subsets with disease activity in patients with SLE.

METHODS

Subject and Study Design

This was a cross-sectional study involving 25 female patients with SLE at Hasan Sadikin Hospital, Bandung. Consent was obtained from each participant. This study was approved by the Board of the Ethics Committee of Padjadjaran University, Bandung. Disease activity scores (SLEDAI-2K) were collected from the medical records of each subject. Subjects were then grouped into inactive (SLEDAI-2K ≤ 4) and active (SLEDAI-2K > 4) groups.

Blood samples were collected via venipuncture and stored in heparin-containing tubes. Hemoglobin, white blood cells, and platelet levels were measured in the laboratory.

Monocyte Characterization

Venous blood samples from the subjects were collected in vacutainer tubes containing lithium and sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). The samples were stored at room temperature for an hour and then measured. Monocytes were identified using multicolor flow cytometry and a panel of monoclonal antibodies consisting of CD14 Alexa Fluor 488 (BioLegend, San Diego, CA, USA), CD16 PE (BioLegend), and HLA-DR PerCP (BioLegend).

Approximately 2000 µL of 0.5% buffer solution was added to a 200 µL heparinized blood sample, followed by vortexing and centrifugation at 1500 rpm for 5 min without a break. The cell suspension was separated, followed by the addition of a mixture of monoclonal antibodies and vortexing of the Fluorescence-Activated Cell Sorting (FACS) buffer diluted solution. The mixture was incubated for 20 min at 2–8°C while covered with aluminum foil. A ten-time diluted red cell lysing buffer (BioLegend) was added to the stained cells and incubated for 12 min. The suspension was vortexed, washed two times using 2000 µL of 0.5% PBA, and suspended in 200 µL of 0.5% PBA. Cells stained with phenotypic markers were scanned and read accordingly using BD Cell Quest Pro Software (Biosciences, San Jose, CA, USA) for 500,000

events. Flow cytometry results were analyzed using FlowJo 10 (Tree Star, California, United State of America). Monocytes were identified using a positive gating strategy according to the phenotypic expression of markers such as CD14, HLA-DR, and CD16.

The monocyte gating strategy (**Figure 1**) started by distinguishing granulocytes using forward scatter and side scatter signals. The positive Boolean gating strategy was implemented on cells with $CD14^{+}HLA\text{-}DR^{+}$ vs $CD14^{-}HLA\text{-}DR^{+}$ phenotype after determining the area of monocytes in leukocytes to identify true monocytes. Monocytes were further characterized based on the expression of CD14 and CD16 into classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and nonclassical ($CD14^{+}CD16^{++}$). Populations of monocyte subsets were represented as a percentage (ratio between absolute monocytes and each monocyte subset).

Statistical Analysis

We initially conducted normality test of our data. Data with normal distribution were presented as mean (±SD), whereas data with skewed distribution were presented as median (range). Differences between groups were evaluated using an unpaired Student t-test and Mann–Whitney test, and area under receiver operating characteristic (ROC) analysis was used to determine the cutoff of monocyte subsets.

RESULTS

The subjects' characteristics and monocyte subsets are shown in **Table 1**.

Compared with individuals without active disease, individuals with active disease had significantly lower mean classical monocyte subsets (71.9% vs 88%, p = 0.008), and higher median intermediate monocytes (29.1% vs 11.1%, p = 0.019). The area under the curve (AUC) of monocyte subsets was determined to identify active disease. The cutoff for classical monocytes was ≤72.2%, AUC = 0.788, p = 0.021, with sensitivity of 66.7% and specificity of 87.5%, whereas for intermediate monocytes, the cutoff was >22.3%, AUC = 0.788, p = 0.014, with sensitivity of 66.7% and specificity of 100% (**Table 2**).

DISCUSSION

The study found that classical monocytes were significantly lower while intermediate monocytes were significantly higher in subjects with active disease compared with those with inactive disease.

Classical monocytes express high levels of CCR2 and CD93 and can phagocytose.

Table 1. Characteristics of Patients with SLE and the Monocyte Subsets

	Inactive (SLEDAI-2K ≤ 4) (n = 16)	Active (SLEDAI-2K > 4) (n = 9)	p
Age (years)	45 ± 11	32 ± 7	0.005*
Duration of illness (months)	79.5 (52.5–133.5)	60 (40.5–110.5)	0.428**
Medications			
Corticosteroid (n, %)	16 (100)	9 (100)	
Mycophenolate mofetil (n, %)	3 (18.8)	3 (33.3)	0.630***
Chloroquine (n, %)	5 (31.3)	1 (11.1)	0.364***
Azathioprine (n, %)	5 (31.3)	4 (44.4)	0.671***
Cyclophosphamide (n, %)	2 (12.5)	1 (11.1)	1.000***
Cyclosporine (n, %)	0 (0.0)	2 (22.2)	0.120***
Hemoglobin (gr/dL)	13 ± 2	11.8 ± 2.6	0.23*
White blood cells (/mm^3)	6,450 (2,920–21,370)	7,430 (4,060–13,540)	0.77**
Monocytes (/mm^3)	567 ± 291	469 ± 108	0.34*
Platelet (/mm^3)	288,813 ± 89,019	304,000 ± 67,337	0.66*
Classical monocytes (%)	88 ± 10.2	71.9 ± 1.7	0.008*
Intermediate monocytes (%)	11.1 (0–22.3)	29.1 (0–49.4)	0.019**
Nonclassical monocytes (%)	0.31 (0.1–10.2)	0.49 (0–2.9)	0.887**

*Unpaired Student t-test; **Mann–Whitney test, ***Fisher's exact test

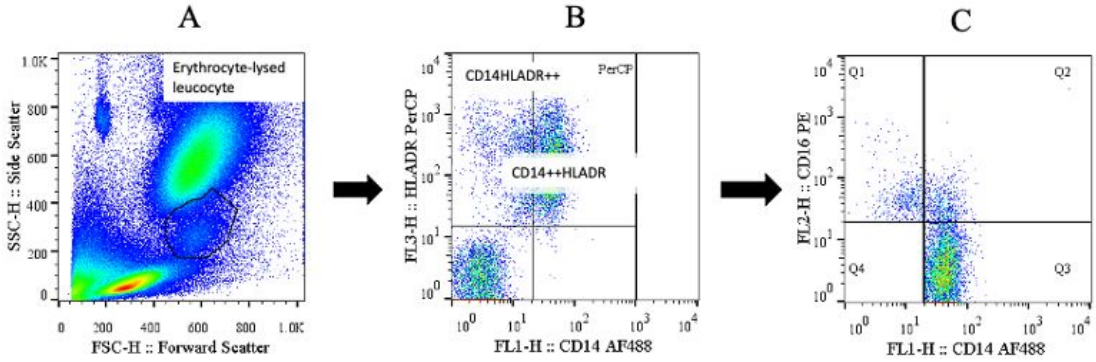

Figure 1. Activated Monocyte Gating Strategy.

(**A**) Identification of monocyte subpopulation in blood. (**B**) Selection of "true" monocytes by gating on CD14-positive and HLA-DR-positive populations. (**C**) The remaining population was further discriminated on a CD14 vs CD16 scatterplot to give three monocyte subsets (Q1-nonclassical subset: $CD14^{+}CD16^{++}$; Q2-intermediate: $CD14^{++}CD16^{+}$; Q3-classical subset: $CD14^{++}CD16^{-}$).

Table 2. Area Under the Curve (AUC) for Monocyte Subsets

	AUC	p-value	Cutoff	Accuracy
Classical monocytes	0.767 (0.557–0.911)	0.021*	≤72.2%	Sensitivity: 66.7% Specificity: 87.5% PPV: 75.0% NPV: 82.4%
Intermediate monocytes	0.788 (0.580–0.924)	0.014*	>22.3%	Sensitivity: 66.7% Specificity: 100.0% PPV: 100.0% NPV: 84.2%

*Significant.
Note: PPV: positive predictive value, NPV: negative predictive value

By contrast, nonclassical monocytes produce large amounts of TNF-α and IL-1β and are considered pro-inflammatory.[6,7] Our study signifies a shift of monocyte population from classical to intermediate because intermediate monocytes emerge from the classical subset.[8] Intermediate monocytes are generally known as an important subset of antigen presentation and $CD8^{+}$ T cell activation. Activation of these cells has been associated with kidney damage and is deemed to expand locally as disease exacerbation occurs.[9] Another possible explanation that might contribute to the finding of lower classical monocytes in active SLE is influenced by interferon-α (IFN-α), which promotes classical monocyte transformation into monocyte-derived dendritic cells (moDCs).[10] These moDCs serve as potent antigen-presenting DCs, particularly in the setting of inflammation.[11] Miyagawa et al. reported that in the murine model, inflammatory moDCs might be essential for autoantibody production and the release of proinflammatory cytokines, such as type I IFN.[12] Type I IFN has been associated with active SLE.[13] T-cell proliferation was enhanced when cocultured with both SLE monocyte subsets, particularly $CD16^{+}$ monocytes, suggesting the potential proinflammatory phenotype of monocyte subsets in patients with SLE compared with healthy controls.[5]

Conflicting studies have been published regarding the variations in monocytes in patients with SLE. Our findings were consistent with a study by Zhu et al. who showed that the frequencies of $CD16^{+}$ (intermediate/nonclassical monocytes) subset were increased while the frequencies of $CD16^{-}$ (classical) monocytes were decreased in patients with SLE. Further analysis revealed that the proportions of $CD16^{+}$ monocytes were also higher in patients with SLE compared with healthy subjects. This monocyte alteration might occur as a result of in vivo cytokine and hormonal environments in SLE, which could lead to the conversion of

$CD16^-$ monocytes into $CD16^+$ monocytes.[5] It was shown that $CD16^+$ monocytes were the producers of proinflammatory cytokines, including TNFα, IL-1, and IL-6.[14] Jin et al. evaluated monocyte gene expression in 15 patients with SLE and 5 healthy donors and showed that the overall expression of classical monocytes was higher in healthy subjects compared with patients with SLE. There was no significant difference in the overall expression of nonclassical monocytes in patients with SLE compared with healthy subjects.[1]

Our findings differed from a previous study that found classical monocytes were significantly higher in 14 patients with active SLE than in 47 patients with inactive SLE (7.64% vs 5.54%, $p = 0.037$, $p < 0.0001$, respectively). Burbano et al. also found that active SLE had higher classical monocytes and lower nonclassical monocytes compared with inactive SLE.[15] Garcia et al. revealed in their study that in patients with severe lupus nephritis, lower levels of nonclassical monocytes were found in peripheral blood.[16]

These differences could be due to variations in enrollment criteria, flow cytometric gating strategies, and sample size. Another possible explanation might be the difference in glucocorticoid doses used in each study. Glucocorticoid treatment decreased the number of $CD16^+$ monocytes (intermediate and nonclassical monocytes) in a dose-related manner.[17] We also concern about possible confounding factors such as age, hemoglobin and other drug.

Jha et al. found that in SLE the absolute monocyte count was lower in the active group than the inactive group (median (IQR) of 329 (228.5) vs. 628 (257)/microliter, $p = 0.001$). The frequency (%) of the intermediate monocyte subset showed a trend towards an increase in active disease (median (IQR) of 15.10% (9.65) vs. 11.85% (8.00), $p = 0.09$). It also had a significant positive correlation to the SLEDAI scores ($r = 0.33$, $p = 0.046$). These findings were consistent with our study.[18]

Although it is well known that classical monocytes are the main participants in the host's defense against infections, mounting evidence from the past ten years suggests that nonclassical or intermediate monocyte subsets play crucial roles in the emergence of SLE. SLE is an immune complex (IC)-mediated autoimmune disorder, and IgG ICs stimulate FcR to activate the monocyte lineage. The process of monocyte differentiation from the classical monocyte subset to the intermediate subset and the subsequent nonclassical subset is sped up by these activation signals.[4]

Active monocyte subsets are related to disease progression. Expansion of the $CD16^+$ monocyte subset (intermediate and nonclassical monocytes) in various diseases has been reported by studies over the past decades, mostly during infection or inflammatory conditions.[5] The typical proportions of total monocytes in healthy subjects are 85% classical monocytes, 5% intermediate monocytes, and 10% nonclassical monocytes.[19] Each monocyte subset has different characteristics and roles within the immune system. Classical monocytes are capable of superior phagocytosis that supports tissue repair and expresses pro-inflammatory proteins, which support inflammatory response. Intermediate monocytes express a higher level of markers for antigen presentation, which makes them responsible for the stimulation and proliferation of T cells.[20] Intermediate monocyte subset is also found to have the highest expression of MHC class I molecules compared with other subsets. This suggests that $CD16^+$ monocytes are capable of activating $CD8^+$ T cells.[20] Nonclassical monocytes are responsible for the proliferation and stimulation of $CD4^+$ T cells. This subset also contains genes that are capable of defining complement components, negatively regulating transcription and pro-apoptosis abilities.[20]

The association between monocyte subsets and SLE disease severity has been reported in previous studies, including the contribution of nonclassical monocytes to the disease.[3,4] Serum levels of anti-dsDNA antibodies were reported to be highly correlated with the percentage of sialoadhesin$^+$ $CD14^{low}CD16^{++}$ nonclassical monocytes in circulation. Nonclassical monocytes are also known to secrete high amounts of IL-1β upon TLR stimulation. Furthermore, it has been reported that antigen presentation by nonclassical monocytes contributes to the activation of T cells and B cells in patients with SLE.[1,4] Hence,

we suggest that monocyte subsets could be a simple hematological parameter to help identify SLE disease activity. We determined a cutoff point of ≤72.2% for the classical subset (66.7% sensitivity and 87.5% specificity, AUC = 0.767 with p = 0.021) and >22.3% for the intermediate subset (67% sensitivity and 100% specificity, AUC = 0.788 with p = 0.014) for active SLE disease.

All our patients received corticosteroids, while the use of other immunosuppressant drugs was not significantly different between patients with inactive or active SLE. We assume that the influence of immunosuppressants on monocyte subset results between the two groups in our study was likely the same. Immunosuppressant drugs have been reported to influence monocytes. Steroid consumption was linked to increased levels of total, $CD14^{++}CD16^{-}$, and $CD14^{++}CD16^{+}$ monocyte counts, but a decrease in $CD14^{+}CD16^{++}$ monocytes. By contrast, the intake of mycophenolate did not have any effect on the counts of monocyte subsets.[21] Cyclosporine has been reported to decrease monocyte counts in patients with lupus nephritis.[22]

The duration of illness in our two groups was not statistically different. There are still controversies regarding the influence of the duration of illness on organ damage in SLE. According to a systematic literature analysis by Sutton et al., organ damage in SLE is often linked to longer disease duration.[23] However, in multivariate analysis, disease duration was comparable between patients with SLE, with or without organ damage.[24]

Our study has several limitations such as the relatively small number of subjects; most of the subjects had already taken several medications, including steroids and immunosuppressants, which might influence hematological parameters. There were no healthy subjects included as controls.

CONCLUSION

Classical and intermediate monocyte subsets could be considered immune cellular markers to identify active SLE.

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COMPETING INTEREST

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

AO, MG, LH, AK: conception, design, data interpretation, drafted and revised the manuscript.
SK: conception, design, data interpretation, revised the manuscript.
LM, NGG: data collection, data interpretation, revised the manuscript.
FF, YM: drafted manuscript, and revised the manuscript.
All authors approved the final version of the manuscript, and all authors are accountable for the accuracy and integrity of all parts of the paper.

REFERENCES

1. Jin Z, Fan W, Jensen MA, Dorschner JM, Bonadurer GF, Vsetecka DM, et al. Single-cell gene expression patterns in lupus monocytes independently indicate disease activity, interferon and therapy. Lupus Science & Medicine. 2017;4(1):e000202.
2. Suszek D, Górak A, Majdan M. Differential approach to peripheral blood cell ratios in patients with systemic lupus erythematosus and various manifestations. Rheumatology International. 2020;40(10):1625-9.
3. Ma W-T, Gao F, Gu K, Chen D-K. The role of monocytes and macrophages in autoimmune diseases: a comprehensive review. Frontiers in immunology. 2019;10:1140.
4. Hirose S, Lin Q, Ohtsuji M, Nishimura H, Verbeek JS. Monocyte subsets involved in the development of

systemic lupus erythematosus and rheumatoid arthritis. International immunology. 2019;31(11):687-96.
5. Zhu H, Hu F, Sun X, Zhang X, Zhu L, Liu X, et al. CD16+ monocyte subset was enriched and functionally exacerbated in driving T-cell activation and B-cell response in systemic lupus erythematosus. Frontiers in immunology. 2016;7:512.
6. Wong KL, Yeap WH, Tai JJY, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: implications for health and disease. Immunologic research. 2012;53:41-57.
7. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nature reviews immunology. 2005;5(12):953-64.
8. Yoshimoto K, Suzuki K, Takei E, Ikeda Y, Takeuchi T. Elevated expression of BAFF receptor, BR3, on monocytes correlates with B cell activation and clinical features of patients with primary Sjögren's syndrome. Arthritis Research & Therapy. 2020;22(1):1-10.
9. Chen P-M, Tsokos GC. The role of CD8+ T-cell systemic lupus erythematosus pathogenesis: an update. Current opinion in rheumatology. 2021;33(6):586.
10. Pan L, Lu M-P, Wang J-H, Xu M, Yang S-R. Immunological pathogenesis and treatment of systemic lupus erythematosus. World Journal of Pediatrics. 2020;16:19-30.
11. Kapellos TS, Bonaguro L, Gemünd I, Reusch N, Saglam A, Hinkley ER, et al. Human monocyte subsets and phenotypes in major chronic inflammatory diseases. Frontiers in immunology. 2019;10:2035.
12. Miyagawa F, Tagaya Y, Ozato K, Horie K, Asada H. Inflammatory monocyte-derived dendritic cells mediate autoimmunity in murine model of systemic lupus erythematosus. Journal of Translational Autoimmunity. 2020;3:100060.
13. Oke V, Gunnarsson I, Dorschner J, Eketjäll S, Zickert A, Niewold TB, et al. High levels of circulating interferons type I, type II and type III associate with distinct clinical features of active systemic lupus erythematosus. Arthritis research & therapy. 2019;21:1-11.
14. Cros J, Cagnard N, Woollard K, Patey N, Zhang S-Y, Senechal B, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. Immunity. 2010;33(3):375-86.
15. Burbano C, Vasquez G, Rojas M. Modulatory effects of CD14+ CD16++ monocytes on CD14++ CD16− monocytes: a possible explanation of monocyte alterations in systemic lupus erythematosus. Arthritis & rheumatology. 2014;66(12):3371-81.
16. Barrera García A, Gómez-Puerta JA, Arias LF, Burbano C, Restrepo M, Vanegas AL, et al. Infiltrating CD16+ are associated with a reduction in peripheral CD14+ CD16++ monocytes and severe forms of lupus nephritis. Autoimmune Diseases. 2016;2016.
17. Sümegi A, Antal-Szalmás P, Aleksza M, Kovács I, Sipka S, Zeher M, et al. Glucocorticosteroid therapy decreases CD14-expression and CD14-mediated LPS-binding and activation of monocytes in patients suffering from systemic lupus erythematosus. Clinical immunology. 2005;117(3):271-9.
18. Jha A, Joseph J, Prabhu SB, Chaudhary A, Yadav B, Mathew J. Utility of peripheral blood monocyte subsets, circulating immune complexes and serum cytokines in assessment of SLE activity: an observational, cross-sectional study. Clinical Rheumatology. 2024;43(1):209-17.
19. Cormican S, Griffin MD. Human monocyte subset distinctions and function: insights from gene expression analysis. Frontiers in immunology. 2020;11:1070.
20. Ożańska A, Szymczak D, Rybka J. Pattern of human monocyte subpopulations in health and disease. Scandinavian journal of immunology. 2020;92(1):e12883.
21. Rogacev KS, Zawada AM, Hundsdorfer J, Achenbach M, Held G, Fliser D, et al. Immunosuppression and monocyte subsets. Nephrology Dialysis Transplantation. 2015;30(1):143-53.
22. Azeez SH. Impact of Cyclosporine A on white blood cell counts and some physiological parameters in renal transplanted patients: A case control study. International Journal of Medical Investigation. 2021;10(1):111-9.
23. Sutton EJ, Davidson JE, Bruce IN, editors. The systemic lupus international collaborating clinics (SLICC) damage index: a systematic literature review. Seminars in arthritis and rheumatism; 2013: Elsevier.
24. Becker-Merok A, Nossent HC. Damage accumulation in systemic lupus erythematosus and its relation to disease activity and mortality. The Journal of Rheumatology. 2006;33(8):1570-7.