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# #1549 Summary



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## Title and Abstract

Title Cytokines, Autoantibodies, and Complements in Active Systemic Lupus Erythematosus Patients from Javanese Population

Abstract

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that has various clinical manifestations. The SLE pathogenesis involves both innate and adaptive immunological components. The system is essentially determined by genetic factors that control certain clinical and serological manifestations. Genetic traits that determine the roles of cytokines, autoantibodies, and complements in SLE vary among ethnicities. The roles of TNF-α, IL-6, anti-C1q, anti-dsDNA, C3, and C4 towards SLE activity need to be evaluated in the Javanese population. This study aimed to determine the correlation of TNF-α, IL-6, anti-C1q antibodies, anti-dsDNA, C3, and C4 with SLE activity. Forty SLE patients were diagnosed based on the American College of Rheumatology (ACR) criteria. Disease activity was measured by the Systemic Lupus Activity Measure (SLAM) index. TNF-α, IL-6, Anti-C1q, and anti-dsDNA levels were measured by ELISA, while MINIMEPH measured C3 and C4. Thirty-nine female and one male patient with SLE were diagnosed according to ACR criteria. The mean of SLAM score, anti-dsDNA, C3, and C4 levels was 20.98±6.7, 224.96±298.6, 68.70±37.08 mg/dL, and 18.75±10.69 mg/dL, respectively. Spearman's correlation test showed a positive correlation between TNF-α ($r$ = 0.971, p<0.001), IL-6 (r=0.835, p<0.001), anti-C1q (r=0.399, p=0.01), and disease activity (SLAM score) by using. The linear regression test for TNF-α, IL-6, anti-C1q, and SLAM showed the strongest association for TNF-α (r=0.891, p<0.000). TNF-α, IL-6, and anti-C1q were correlated to disease activity in SLE patients from the Javanese population.

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Keywords Systemic lupus erythematosus, tumor necrosis factor-α, interleukin-6, biomarkers
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## References

References

[1] Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. J Clin Pathol. 562003. p. 481-90.

[2] Tackey E, Lipsky PE, Illei GG. Rationale for interleukin-6 blockade in systemic lupus erythematosus. Lupus. 2004;13(5):339-43.

[3] Romero-Diaz J, Isenberg D, Ramsey-Goldman R. Measures of adult systemic lupus erythematosus: updated version of British Isles Lupus Assessment Group (BILAG 2004), European Consensus Lupus Activity Measurements (ECLAM), Systemic Lupus Activity Measure, Revised (SLAM-R), Systemic Lupus Activity Questionnaire for Population Studies (SLAQ), Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI). Arthritis care & research. 2011;63 Suppl 11:S37-46.

[4] Umare V, Pradhan V, Nadkar M, Rajadhyaksha A, Patwardhan M, Ghosh KK, et al. Effect of Proinflammatory Cytokines (IL-6, TNF-α, and IL-1β) on Clinical Manifestations in Indian SLE Patients. Mediators of Inflammation. 2014;2014:8.

[5] Sabry A, Sheashaa H, El-Husseini A, Mahmoud K, Eldahshan KF, George SK, et al. Proinflammatory cytokines (TNF-alpha and IL-6) in Egyptian patients with SLE: its correlation with disease activity. Cytokine. 2006;35(3-4):148-53.

[6] McCarthy EM, Smith S, Lee RZ, Cunnane G, Doran MF, Donnelly S, et al. The association of cytokines with disease activity and damage scores in systemic lupus erythematosus patients. Rheumatology (Oxford, England). 2014;53(9):1586-94.

[7] Giles BM, Boackle SA. Linking complement and anti-dsDNA antibodies in the pathogenesis of systemic lupus erythematosus. Immunologic research. 2013;55(1-3):10-21.

[8] Illei GG, Tackey E, Lapteva L, Lipsky PE. Biomarkers in systemic lupus erythematosus: II. Markers of disease activity. Arthritis and rheumatism. 2004;50(7):2048-65.

[9] Maury CPJ, Teppo AM. Tumor necrosis factor in the serum of patients with systemic lupus erythematosus. Arthritis & Rheumatism. 1989;32(2):146-50.

[10] Panafidina TA, Popkova TV, Novikov AA, Nasonov EL. AB0630 Expression of tnf-A and il-6 in systemic lupus erythematosus: relationship with disease activity. Annals of the Rheumatic Diseases. 2018;77(Suppl 2):1462-3.

[11] Idborg H, Eketjäll S, Pettersson S, Gustafsson JT, Zickert A, Kvarnström M, et al. TNF-α

and plasma albumin as biomarkers of disease activity in systemic lupus erythematosus. Lupus Science & Medicine. 2018;5(1).

[12] Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. Science (New York, NY). 2002;296(5573):1634-5.

[13] Palucka AK, Blanck JP, Bennett L, Pascual V, Banchereau J. Cross-regulation of TNF and IFN-α in autoimmune diseases. Proc Natl Acad Sci U S A. 1022005. p. 3372-7.

[14] Gabay C, Cakir N, Moral F, Roux-Lombard P, Meyer O, Dayer JM, et al. Circulating levels of tumor necrosis factor soluble receptors in systemic lupus erythematosus are significantly higher than in other rheumatic diseases and correlate with disease activity. The Journal of rheumatology. 1997;24(2):303-8.

[15] Garaud S, Le Dantec C, Jousse-Joulin S, Hanrotel-Saliou C, Saraux A, Mageed RA, et al. IL-6 Modulates CD5 Expression in B Cells from Patients with Lupus by Regulating DNA Methylation. The Journal of Immunology. 2009;182(9):5623-32.

[16] Charles P, Elliott MJ, Davis D, Potter A, Kalden JR, Antoni C, et al. Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-alpha therapy in rheumatoid arthritis. Journal of immunology (Baltimore, Md : 1950). 1999;163(3):1521-8.

[17] Jacob N, Stohl W. Cytokine disturbances in systemic lupus erythematosus. Arthritis research & therapy. 2011;13(4):228.

[18] Enocsson H, Sjowall C, Kastbom A, Skogh T, Eloranta ML, Ronnblom L, et al. Association of serum C-reactive protein levels with lupus disease activity in the absence of measurable interferon-alpha and a C-reactive protein gene variant. Arthritis & rheumatology (Hoboken, NJ). 2014;66(6):1568-73.

[19] Richards HB, Satoh M, Shaw M, Libert C, Poli V, Reeves WH. Interleukin 6 Dependence of Anti-DNA Antibody Production: Evidence for Two Pathways of Autoantibody Formation in Pristane-induced Lupus. The Journal of Experimental Medicine. 1998;188(5):985-90.

[20] Fu SM, Dai C, Zhao Z, Gaskin F. Anti-dsDNA Antibodies are one of the many autoantibodies in systemic lupus erythematosus. F1000Research. 2015;4(F1000 Faculty Rev).

[21] Okamura M, Kanayama Y, Amastu K, Negoro N, Kohda S, Takeda T, et al. Significance of enzyme linked immunosorbent assay (ELISA) for antibodies to double stranded and single stranded DNA in patients with lupus nephritis: correlation with severity of renal histology. Annals of the Rheumatic Diseases. 1993;52(1):14-20.

[22] MacGowan JR, Ellis S, Griffiths M, Isenberg DA. Retrospective analysis of outcome in a cohort of patients with lupus nephritis treated between 1977 and 1999. Rheumatology (Oxford, England). 2002;41(9):981-7.

[23] López-Hoyos M, Cabeza R, Martínez-Taboada VM, Crespo J, SanSegundo D, Blanco R, et al. Clinical disease activity and titers of anti-dsDNA antibodies measured by an automated immunofluorescence assay in patients with systemic lupus erythematosus. Lupus. 2005;14(7):505-9.

[24] Sturfelt G, Truedsson L. Complement in the immunopathogenesis of rheumatic disease. Nature reviews Rheumatology. 2012;8(8):458-68.

[25] Nguyen CP, Cao VV, Feher J, Gergely P. Correlation between the levels of antinuclear antibodies, anti-DNA antibodies, and complement in systemic lupus erythematosus. Acta medica Hungarica. 1988;45(2):145-59.

[26] Narayanan K, Marwaha V, Shanmuganandan K, Shankar S. Correlation between Systemic Lupus Erythematosus Disease Activity Index, C3, C4 and Anti-dsDNA Antibodies. Medical Journal, Armed Forces India. 2010;66(2):102-7.

[27] Einav S, Pozdnyakova OO, Ma M, Carroll MC. Complement C4 is protective for lupus disease independent of C3. Journal of immunology (Baltimore, Md : 1950). 2002;168(3):1036-41.

[28] Walport MJ. Complement and systemic lupus erythematosus. Arthritis research. 2002;4 Suppl 3:S279-93.

[29] Moura CG, Lima I, Barbosa L, Athanazio D, Reis E, Reis M, et al. Anti-C1q antibodies: association with nephritis and disease activity in systemic lupus erythematosus. Journal of Clinical Laboratory Analysis. 2009;23(1):19-23.

[30] Lood C, Gullstrand B, Truedsson L, Olin AI, Alm GV, Rönnblom L, et al. C1q inhibits immune complex–induced interferon-α production in plasmacytoid dendritic cells: A novel link between C1q deficiency and systemic lupus erythematosus pathogenesis. Arthritis & Rheumatism. 2009;60(10):3081-90.

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**RESEARCH**

# CYTOKINES, AUTOANTIBODIES, AND COMPLEMENTS IN ACTIVE SLE PATIENTS FROM JAVANESE POPULATION


## ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease that has various clinical manifestations. The SLE pathogenesis involves both innate and adaptive immunological components. The system is essentially determined by genetic factors that controls over certain clinical and serological manifestations. Genetical traits that determine the roles of cytokines, autoantibodies, and complements in SLE vary among ethnicities. The roles of TNF-α, IL-6, anti-C1q, anti-dsDNA, C3, and C4 towards SLE activity need to be evaluated in Javanese population. To determine the correlation of TNF-α, IL-6, anti-C1q antibodies, anti-dsDNA, C3, and C4 with the activity of SLE. Forty SLE patients were diagnosed using American College of Rheumatology criteria. Disease activity is measured by Systemic Lupus Activity Measure (SLAM) index. TNF-α, IL-6, Anti-C1q, and anti-dsDNA levels were measured by ELISA, whilst C3 and C4 were measured by MINIMEPH. Thirty-nine females and one male patients with SLE were diagnosed according to ACR criteria. The mean SLAM score was 20.98±6.7, anti-dsDNA 224.96±298.6, C3 68.70±37.08 mg/dL, and C4 18.75±10.69 mg/dL. We found a positive correlation between TNF-α ($r$ = 0.971, p<0.001), IL-6 (r = 0.835, p<0.001), anti-C1q (r=0.399, p=0.01), and disease activity (SLAM score) by using Spearman's correlation test. The linear regression test for TNF-α, IL-6, anti-C1q and SLAM showed the strongest association for TNF-α (r=0.891, p<0.000). TNF-α, IL-6 and anti-C1q were correlated to disease activity in SLE patients from Javanese population.



**Keywords:** Systemic Lupus Erythematosus, Tumor Necrosis Factor-α, Interleukin-6, Biomarkers


**Commented [ui1]:** Kalimat belum lengkap

**Commented [ui2]:** (ACR)

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## INTRODUCTION

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Systemic lupus erythematosus (SLE) is a chronic systemic inflammatory disease caused by autoimmune mechanism. SLE begins with loss of immunological tolerance that initiates an auto-reaction from effector T-cells and leads to immunological response to antigens. This process involves various mechanisms including autoantibody production, complement activation, immune complex formation, and leukocyte infiltration. Cytokine production in SLE patients certainly differ from that in healthy individuals. Cytokines are accountable in regulating SLE activity and in different organs involvement.[1]

The presence of self-antigens triggers the activation of antigen-presenting cells (APC), T-cells, and B-cells through biochemical signal from cytokines such as IL-6 and TNF-α. TNF-α is a pleiotropic cytokine that presumably causes apoptosis, activates dendritic cells, and stimulates IL-6 secretion. Studies reported that IL-6 stimulates both T-cells differentiation to become effector cells and B-cell differentiation to synthesize anti-dsDNA and anti-C1q. This feature causes hyper-response towards autoantibodies and organ inflammation.[1, 2]

Generally, anti-C1q, C3, and C4 are found at low level in SLE patients. These complements normally act as antigen clearance or immunological complex in order to prevent overactivity of immune response. Complement deficiency is one of the reasons SLE patients are susceptible to autoimmune reaction. A study on patients without hereditary C1q deficiency reported that anti-C1q was found in active SLE. C3 and C4 level also decreases due to high clearance of elevated immune complexes.[2]

Previous studies reported various results for assessing disease activity possibly due to the variety of ethnic groups. It is crucial to evaluate the roles of various cytokines, antibodies, and autoantibodies to disease activity in SLE. This study aims to correlate TNF-α, IL-6, anti-C1q antibodies, anti-dsDNA, and C3 & C4 to disease activity, measured by SLAM index, in active SLE patients from Javanese population.

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## METHODS

### Study Patients

This is a cross-sectional study conducted in Department of Internal Medicine, Dr. Soetomo General Hospital, Surabaya between January and June 2013. All data was collected from the medical records of newly diagnosed SLE patients who met four of the revised 1997 ACR criteria. Each patient who consented to participate in this study filled out the informed consent. This study has been approved by the Ethnic Review Committee (Approval number 312/Panke.KKE/I/2013). Non-probability consecutive sampling technique was used to enroll subjects, considering the confounding and bias factors. Infectious patients were excluded if their blood/urine/sputum culture indicates micro-organism infections. Patients who showed symptoms overlap with other connective tissue diseases were also not included in this study.

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Forty patients with age range of 30±8.1 years consisting of 39 female and one male newly-diagnosed SLE patients were included in this study. We used ACR criteria for SLE diagnosis and found four out of the 11 criteria.

### SLAM Index

Systemic Lupus Activity Measure (SLAM) index is a measuring tool for assessing SLE disease activity by weighting each parameter between 0-3. The total score is a sum of all parameters and it ranges from 0 to 86. Higher score indicates more active or severe illness. SLAM has an interlined reliability index of 0.86 and an interval of reliability of 0.73.[3]

### Anti-C1q Autoantibody Level

Anti-C1q and anti-dsDNA levels were measured by ELISA method. The examination was performed according to standard. All reagents and blood samples were prepared according to instruction, then samples were added to microwells. Later, protein biotin preparations were added in each microwell and incubated at room temperature. After we washed them, we added the preparations with Streptavidin-Peroxidase Conjugate then we incubated it at room temperature. We washed it once more before we put Chromogen Substrate to each well then we incubated it at room temperature. We added stop solution before we read the result with flowcytometry in anti-C1q with levels of positive kit >10 U/ml using Anti-C1q Elisa kit (ORGENTEC Diagnostika GmbH, Mainz, Germany), 0-200U/ ml.

Commented [ui5]: standard apa?

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**Complement Level**

Complement from patient's serum was measured by MINIMEPH human complement. It could detect C4 level that ranges between 0.7 and 27 g/L and C3 of 0.275-4.44 g/L.

**TNF-α Serum Level**

Levels of TNF-α in serum were analyzed with ELISA method, then the result was read in ELISA reader in the form of optical density. The result was converted to pg/mL according to the instructions in the ELISA kit (Bender MedSystems, Vienna-Austria) that could detect >2 pg/mL.

**IL-6 Serum Level**

ELISA method was used to examine the IL-6 level. The reagents used was Human IL-6 (Quantikine® ELISA Human IL-6 Immunoassay, R&D System, Inc. Catalog D6050) and then was read with ELISA reader. Normal serum level of IL-6 is > 3.12 pg/ml. This reagent is able to detect IL-6 ≥0.7 pg / ml.

Before the examination, microwells were stored and wrapped in aluminum foil at 2-8$^{o}$C, then washed with wash buffer with concentration as much as 400 µL twice. After that, we let them stand for 10-15 seconds and dried it with absorbance. The dry microwells must not be placed upside down. 50 µL biotin-conjugate was dripped into each well, 50 µL serum was added from the samples, and the microwells were incubated for 2 hours at room temperature (20° C). Then, it was covered with film adhesion and incubated at room temperature (18-25$^{o}$C).

After 2 hours, the adhesive film cover was removed and the solution from the wells was disposed. Microwells were washed three times using 400 µL wash buffer solution before 100 Streptavidin-HRP were added at all wells, covered with adhesive film, and incubated at room temperature (18-25°C) for an hour. After that, microwells were washed three times with 400 µL wash buffer solution again.

Furthermore, all microwells were added with 100 µL TMB substrate, then covered with aluminum foil to avoid light and incubated at room temperature (18-25$^{o}$C) for 10 minutes. It was recommended to provide 100 µL stop solution in the dark if the wells with the highest concentration of the standard turned dark blue or dark. Finally, the results were generated within an hour by using spectrophotometer with 450 nm wavelength.

**Data Analysis**

We used a computer software for statistical test SPSS for data analysis. Mean and standard deviation were determined on the quantitative data. This study aimed to test the

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hypothesis of whether or not serum IL-6, TNFα, C3, C4 and anti-dsDNA were correlated to disease activity (SLAM score). We performed data normality test using Kolmogorov-Smirnov test before determining the correlation. The interpretation of correlation test results was based on p-value, correlation strength, and direction of correlation. In this study, the correlation between IL-6, TNF-α, C3, C4 and anti-dsDNA serum with SLAM score resulted in p=0.000 (less than 0.05, α=5%). The correlation strength (r) of p=0.00-0.199 was interpreted as very weak. The p-value of 0.20-0.399 was interpreted as weak, the p-value of 0.40-0.599 was considered as a moderate correlation, the p-value of 0.60-0.799 was interpret as a strong correlation, and p-value of 0.80 to 1.00 was considered as a very strong correlation. Multivariate test (linear regression / Pearson's correlation) was performed to determine the correlation strength of SLAM with TNF-α, IL-6 and anti-C1q autoantibodies.

**Commented [ui6]:** P value or r value?
Bedakan dengan p value less than 0.05

**RESULTS AND DISCUSSION**

The subjects' characteristics were shown in Table 1. The highest education level recorded was elementary school and almost all the subjects came from low economic level society. Weight loss and hair loss are the two most prominent symptoms. Clinical manifestations such as musculoskeletal disorders, hematology and nephropathy were prominent (Table 2). The results from laboratory tests and SLAM index showed that out of forty newly diagnosed SLE patients in Dr. Soetomo General Hospital Surabaya, 97.5% of them were women with an average age of 30 ± 8.1 years, mean SLAM score of 20.98 ± 6.7, TNF-α of 3.9±1.15 pg/ml, IL-6 of 49.8±73.53 pg/ml, anti-dsDNA level of 224.96 ± 298.6 IU/ml, anti-C1q of 42.18±52.16 IU/ml, C3 level of 68.70 ± 37.08 mg/dl, and C4 level of 18.75 ± 10.69 mg/dl (Table 3).

**Table 1.** Characteristics of Systemic Lupus Erythematosus patients

| Characteristics | | Quantity | Percentages (%) |
|---|---|---|---|
| Age | Mean (30) ± 8.1 year<br>Range (16 – 47 year) | | 100 |
| Sex | Male | 1 | 2.50 |
| | Female | 39 | 97.50 |
| Level of Education | Bachelor | 4 | 10 |
| | Senior High School | 15 | 36.20 |
| | Junior High School | 10 | 25 |
| | Elementary School | 11 | 10 |
| Weight Loss | | 27 | 67.50 |
| Fatigue | | | |
| Fever | | 18 | 45 |
| Hair Loss | | 33 | 82.50 |

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|---|---|---|---|
| Hemoglobin | < 5 G/dl | 8 | 20 |
| | 5-10 G/dl | 26 | 65 |
| | > 10 G/dl | 6 | 15 |
| C3 | < 85 mg/dl (n: 85-180 mg/dl) | 26 | 65 |
| C4 | < 10 mg/dl (n: 10-40 mg/dl) | 13 | 32.5 |
| Anti-C1q | ≥10 U/ml | 33 | 77.5 |
| | <10 U/ml | 9 | 22.5 |

**Table 2.** Patients' Clinical Features based on 1997 ACR Criteria

| American College Rheumatology Criteria | Quantity | Percentage |
|---|---|---|
| Malar rash | 23 | 57.5 |
| Discoid rash | 8 | 20 |
| Oral ulcer | 24 | 60 |
| Photosensitivity | 7 | 17.5 |
| Arthritis | 32 | 80 |
| Serositis | 12 | 30 |
| Nephritis | 17 | 42.5 |
| Neuropsychiatric disorders | 6 | 15 |
| AIHA | 2 | 5 |
| Leukopenia | 9 | 25 |
| Lymphopenia | 35 | 87.5 |
| Trombocytopenia | 21 | 52.5 |
| ANA (positive ≥ 20 unit) | 27 | 67.5 |
| Anti-dsDNA (positive ≥ 92.6 Unit/ ml) | 17 | 42.5 |

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**Table 3.** Mean Level of Cytokines, Autoantibodies, Complements, and SLAM score

| | Minimum | Maximum | Mean±SD |
|---|---|---|---|
| TNF-α | 2.6 | 7.7 | 3.9±1.15 |
| IL-6 | 3.10 | 355.10 | 49.8±73.53 |
| Anti-dsDNA | 6 | 1403 | 224.96±298.6 |
| Anti-C1q | 1.0 | 214.7 | 42.18±52.16 |
| C3 | 22 | 149 | 68.70±37.08 |
| C4 | 5 | 45 | 18.75±10.69 |
| SLAM | 7 | 35 | 20.98±6.7 |

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Correlation test was performed using Spearman's correlation of cytokines, autoantibodies, and complement to disease activity (Table 4). TNF-α (r=0.971, p<0.000), IL-6 (r=0.835, p<0.000), and anti-C1q (r=0.399 p<0.011) showed significant correlation to SLAM index (disease activity). Multivariate test was performed using linear regression to determine the

strength of association between TNF-α, IL-6, anti-C1q with SLAM index. TNF-α and SLAM appeared to have the strongest association (r=0.891, p <0,000) (Table 5).

**Table 4.** Spearman's correlation of cytokines, autoantibodies, and complements with disease activity (SLAM index)

| Antibodies | SLAM index | |
|---|---|---|
| | ***p*** | **r** |
| TNF-α | 0.000 | 0.971 |
| IL-6 | 0.000 | 0.835 |
| Anti-C1q | 0.011 | 0.399 |
| C3 | -0.242 | 0.132 |
| C4 | -0.174 | 0.283 |
| Anti-dsDNA | 0.124 | 0.447 |

**Table 5.** Strength of Association between SLAM index with TNF-α, IL-6, and Anti-C1q using Linear Regression (Pearson's correlation)

| Antibodies | SLAM | |
|---|---|---|
| | ***p*** | **r** |
| TNF-α | 0.000 | 0.891 |
| IL-6 | 0.000 | 0.608 |
| Anti-C1q | 0.007 | 0.389 |

We found a strong correlation of SLAM score with TNF-α (r=0.971, p<0.000) (Table 4) with a mean level of TNF-α of 3.9±1.15 mg/dl. Both also showed the strongest association among others (r=0.891, p<0.000) (Figure. 1). This result varies from previous studies of TNF-α on other ethnic groups such as Caucasian (12.63 mg/dl), Indian (63.00±67.28 mg/dl), and Brazilian (2.18 IU/dl).[4] What might cause these differences hasn't yet been known but genetic trait and environmental condition might be some of them. Furthermore, Sabry (2006) reported that the elevation of TNF-α level had the strongest correlation to lupus activity (SLEDAI index), especially lupus nephritis.[5] McCharty (2014) also reported a significant TNF-α elevation associated to disease activity (SLEDAI index) with high five-year-period severity.[6] This study showed a significant association between TNF-α and disease activities (organ-damage manifestations) such as arthritis, serositis, lupus nephritis, NPSLE, and anemia.

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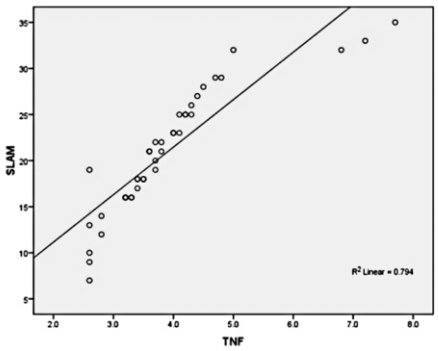


**Figure 1.** Strength of Association between TNF-α and Disease Activity

In this study, TNF-α level represented the expressions of cytokines, while anti-C1q was correlated to disease activity. On the other hand, complements and anti-dsDNA were not correlated disease activity. The decrease of C3 and C4 levels can lead to more severe disease activity. Complements activity may vary among individuals and they do not represent an ongoing immune-response activity that well. This result hints to the complexity of SLE pathogenesis. According to the autoantibody theory, the formation of immune complexes triggers complement activation which can cause inflammation to multiple organs.[7]

Any inflammatory cytokine under normal condition is induced by an anti-inflammatory cytokine. The cytokine imbalance triggers SLE to become more progressive.[8] A study by Tackey (2004) reported that some cytokines were associated with disease activity. Many other studies reported that cytokines hold a major role in regulating the immune system. Cytokines in SLE play a role in the formation of autoantibodies and tolerance processes. In addition, cytokines also affect the production of autoantibodies. The last decade studies reported that several cytokines were involved in SLE pathogenesis. Elevated IL-6 enhances CRP and serum amyloid proteins that are involved in the tolerance process of apoptotic fragments. Both IL-6 and TNF-α induce the secretion of acute phase proteins which leads to the discharge of autoantibodies to systemic circulation.[2]

It has been established that TNF-α holds a major role in the pathogenesis of Rheumatoid Arthritis (RA), therefore the main goal of RA therapy is to maintain normal TNF-α level in order to control disease progressivity. Meanwhile, studies on TNF-α role for SLE activity have been showing inconsistencies. Maury CP & Teppo (1989) obtained TNF-α levels of 14 pg/L in active

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SLE with no significant difference between those with lupus nephritis and not. They obtained TNF-α levels of 24 pg/L in active RA from patients' synovial fluid compared with serum TNF-a in patient with SLE. Active SLE patients showed high levels of TNF-α that are almost identical with those in RA taken from synovial fluid. TNF-α also has similar roles in RA as proinflammatory cytokines.[9] Compared to that study, Panafidina, *et al.* (2018) reported that higher IL-6 and lower TNF-α levels as compared to healthy controls and there was no correlation of disease activity to TNF-α and IL-16. They also reported that SLE patients during active phase had decreased TNF-α level. They suspected that TNF-α in SLE was an immunoregulator.[10] Idborg, *et al.* (2018) reported that TNF-α correlated with disease activity and they suggested that TNF-α could be used for biomarkers.[11]

In our study, TNF-α level was at 3.9 ± 1.15pg/L. Idborg reported a TNF-α of 4.5,[11] which was similar to our study. Although TNF-a was not as high as in previous studies, we suspected that TNF-a still has inflammatory effect in active SLE. It was proven by the fact that 90% of subjects suffer lymphopenia. Our study found a prominent lymphopenia which was suspected as a condition in apoptotic T-cells induced by TNF-α. It was in accordance with the fact that TNF-α has a role in inducing apoptosis.[12] According to previous studies, TNF-α induced autoantibody synthesis against cell component and that process was enhanced by interferon alpha (IFN-α). It was proved by a study Palucka, *et al.* (2005) that activity of TNF-a depends on INF-α level[13] and both were simultaneously increased in active SLE.[14]

IL-6 is a pleiotropic cytokine, that is mostly produced by macrophage and holds an important role in the regulation of the immune and inflammatory systems.[2] Garaud, *et al*. (2009) reported that IL-6 increased CD5 expression in B cells thus IL-6 increased the number of B cells population.[15] In addition, IL-6 could induce TNF-α so that each cytokines affected each other.[16] The cytokines level in each patient was different, presumably due to differences in clinical manifestations of SLE.[17] IL-6 and disease activity in this study were well correlated ($r=0.835$, $p<0.000$) (Table 4) with an average IL-6 level of 49.8±73.53 pg/ml (Table 3) and showed a moderate strength of association (Figure. 2). The mean level was slightly lower than Indian ethnicity (63.00±67.28 pg/dl), but way higher than other group like Brazilian (1.5 pg/ml) or a study on multiethnicities (1.64 pg/ml).[4] It's still unknown to what might cause these various results.

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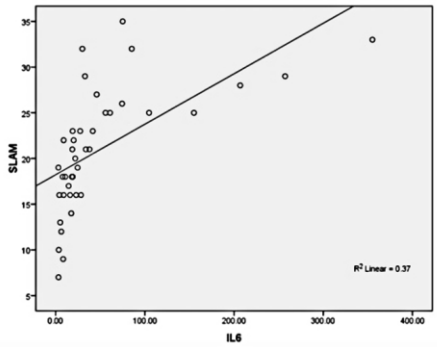


**Figure 2.** Strength of Association between IL-6 and Disease Activity

According to a study by Enocsson (2014), elevation of IL-6 level was parallel to disease activity in SLE (r=0.389 P<0.013).[18] William (2005) stated that IL-6 was not correlated to CRP in lupus and Richard (1998) in an animal study reported that IL-6 induced the synthesis of anti-dsDNA. The formation of anti-dsDNA was highly dependent on IL-6.[19] IL-6 allegedly depicts a localized inflammation, especially in kidneys. The role of antibodies in inflammation process is most apparent in the kidneys where antibodies activate complement and cause inflammation, but this process is systemically undetected.

It has been reported that the association of anti-dsDNA to disease activity and its ability to predict SLE recurrence are very limited and anti-dsDNA was not the only antibody related to SLE activity. The reason of anti-dsDNA cannot be used as a guide for determining SLE disease activity because it depends on assay technique. Positive anti-dsDNA does not always mean the occurrence of SLE and negative anti-dsDNA does not exclude SLE diagnosis.[20] Anti-dsDNA is a very specific antibody marker for active SLE, found abundantly in renal tissue.[8] A study on animal showed that the incidence of glomerulonephritis was related to anti-dsDNA level.[21] A cohort study reported that high level of anti-dsDNA is associated with the severity and the progressivity of kidney damage.[22] M Lo'pez-Hoyos, *et al.* (2005) obtained that anti-dsDNA level in active lupus was 59.3+8.7 IU/mL (measured by EliA dsDNA).[23] In this study found that the mean anti-dsDNA level was 224.96±298.6 IU/mL, which was higher than other previous studies. These differences are suspected to be related to genetic factors.

Complements' role in SLE was complex. A complement activation causes a decreased in C3 and C4 levels in active SLE but their concentration will rapidly rise to its initial level after the

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activation process is complete. In addition, C4 rises back its normal level faster than C3 in order to prevent further complement activation. It is widely known that complement holds a major role in inflammation and organ damage associated with an autoimmune or immune complex mediated disease.[24] A study by Nguyen (1988) in active SLE patients obtained that C3 and C4 average levels were 37.50 mg/dl and 22.29 mg/dl respectively, therefore C3 and C4 could be used as diagnostic tools to assess disease activity.[25] Narayanan (2010) also reported a C3 average level of 81.08 mg/dl and a C4 level of 15.05 mg/dl.[26] Furthermore, a study by Einav (2002) reported that C4 was protective against lupus but at the same time was also strongly associated with C3 in terms of its relation to disease activity.[27] Meanwhile, we found that the mean of C3 level was 68.70 ± 37.08 mg/dl and C4 was 18.75 ± 10.69 mg/dl. In lupus, complements exhibit duality in which they are related to tissue damage, but on the other hand they also protect tissues against inflammation due to immune complexes.

Anti-C1q is a component in the classic pathway of complement activation that generally serves to neutralize immune complexes,[28] and correlated to risks and activity of lupus.[29] Anti-C1q was suspected to have other biological functions that play a role in facilitating and inhibiting the pathogenesis of lupus. Lood, *et al.* (2009) reported the novel function of anti-C1q in regulating immune complex that induces the production of IFN-α and other cytokines in the plasmacytoid dendritic cells. Anti-C1q allegedly has various roles in the pathogenesis of SLE and its clinical manifestations.[30] Anti-C1q level in this study was shown to be well correlated with SLAM ($r=0.399$ $p<0.011$) (Table 4) with mean level of 42.18±52.16 IU/ml. We also found that anti-C1q had a positive association to disease activity ($r=0.389$, $p=0.007$) (Figure. 3) although it wasn't as strong as TNF-α or IL-6 (Table 5). It was in accordance to a study by Moura, *et al.* (2009) that reported that the elevation of anti-C1q level was followed by the worsening of SLE activity.[29]

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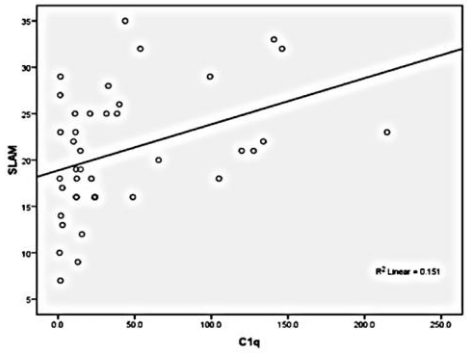


**Figure 3.** Strength of Association between Anti-C1q and Disease Activity

## Conclusion and Suggestion

Cytokines showed an undisputable role in inflammation process and autoimmune disease. Their pleiotropic effect made their components have both synergistic or antagonistic to each other. Not only cytokines, autoantibodies and complements were also involved in inflammation process and tissue injury through complement-activation cascade. However, it was difficult to determine cytokines, autoantibodies, and complements that are involved in the pathogenesis of SLE. It raised a question on immunological components integrate as immune system. In conclusion, IL-6, TNF-α, and anti-C1q were correlated to disease activity in SLE patients from Javanese population, although their roles to the pathogenesis of certain clinical manifestations remain unclear. Therefore, further cohort study is necessary to evaluate the role of cytokines in both active and inactive diseases.

**Commented [ui18]:** Saran masuk dlm discussion

## References

[1] Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. J Clin Pathol. 562003. p. 481-90.

[2] Tackey E, Lipsky PE, Illei GG. Rationale for interleukin-6 blockade in systemic lupus erythematosus. Lupus. 2004;13(5):339-43.

[3] Romero-Diaz J, Isenberg D, Ramsey-Goldman R. Measures of adult systemic lupus erythematosus: updated version of British Isles Lupus Assessment Group (BILAG 2004), European Consensus Lupus Activity Measurements (ECLAM), Systemic Lupus Activity Measure, Revised (SLAM-R), Systemic Lupus Activity Questionnaire for Population Studies (SLAQ), Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI). Arthritis care & research. 2011;63 Suppl 11:S37-46.

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[4] Umare V, Pradhan V, Nadkar M, Rajadhyaksha A, Patwardhan M, Ghosh KK, et al. Effect of Proinflammatory Cytokines (IL-6, TNF-α, and IL-1β) on Clinical Manifestations in Indian SLE Patients. Mediators of Inflammation. 2014;2014:8.

[5] Sabry A, Sheashaa H, El-Husseini A, Mahmoud K, Eldahshan KF, George SK, et al. Proinflammatory cytokines (TNF-alpha and IL-6) in Egyptian patients with SLE: its correlation with disease activity. Cytokine. 2006;35(3-4):148-53.

[6] McCarthy EM, Smith S, Lee RZ, Cunnane G, Doran MF, Donnelly S, et al. The association of cytokines with disease activity and damage scores in systemic lupus erythematosus patients. Rheumatology (Oxford, England). 2014;53(9):1586-94.

[7] Giles BM, Boackle SA. Linking complement and anti-dsDNA antibodies in the pathogenesis of systemic lupus erythematosus. Immunologic research. 2013;55(1-3):10-21.

[8] Illei GG, Tackey E, Lapteva L, Lipsky PE. Biomarkers in systemic lupus erythematosus: II. Markers of disease activity. Arthritis and rheumatism. 2004;50(7):2048-65.

[9] Maury CPJ, Teppo AM. Tumor necrosis factor in the serum of patients with systemic lupus erythematosus. Arthritis & Rheumatism. 1989;32(2):146-50.

[10] Panafidina TA, Popkova TV, Novikov AA, Nasonov EL. AB0630 Expression of tnf-A and il-6 in systemic lupus erythematosus: relationship with disease activity. Annals of the Rheumatic Diseases. 2018;77(Suppl 2):1462-3.

[11] Idborg H, Eketjäll S, Pettersson S, Gustafsson JT, Zickert A, Kvarnström M, et al. TNF-α and plasma albumin as biomarkers of disease activity in systemic lupus erythematosus. Lupus Science & Medicine. 2018;5(1).

[12] Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. Science (New York, NY). 2002;296(5573):1634-5.

[13] Palucka AK, Blanck JP, Bennett L, Pascual V, Banchereau J. Cross-regulation of TNF and IFN-α in autoimmune diseases. Proc Natl Acad Sci U S A. 1022005. p. 3372-7.

[14] Gabay C, Cakir N, Moral F, Roux-Lombard P, Meyer O, Dayer JM, et al. Circulating levels of tumor necrosis factor soluble receptors in systemic lupus erythematosus are significantly higher than in other rheumatic diseases and correlate with disease activity. The Journal of rheumatology. 1997;24(2):303-8.

[15] Garaud S, Le Dantec C, Jousse-Joulin S, Hanrotel-Saliou C, Saraux A, Mageed RA, et al. IL-6 Modulates CD5 Expression in B Cells from Patients with Lupus by Regulating DNA Methylation. The Journal of Immunology. 2009;182(9):5623-32.

[16] Charles P, Elliott MJ, Davis D, Potter A, Kalden JR, Antoni C, et al. Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-alpha therapy in rheumatoid arthritis. Journal of immunology (Baltimore, Md : 1950). 1999;163(3):1521-8.

[17] Jacob N, Stohl W. Cytokine disturbances in systemic lupus erythematosus. Arthritis research & therapy. 2011;13(4):228.

[18] Enocsson H, Sjowall C, Kastbom A, Skogh T, Eloranta ML, Ronnblom L, et al. Association of serum C-reactive protein levels with lupus disease activity in the absence of measurable interferon-alpha and a C-reactive protein gene variant. Arthritis & rheumatology (Hoboken, NJ). 2014;66(6):1568-73.

[19] Richards HB, Satoh M, Shaw M, Libert C, Poli V, Reeves WH. Interleukin 6 Dependence of Anti-DNA Antibody Production: Evidence for Two Pathways of Autoantibody Formation in Pristane-induced Lupus. The Journal of Experimental Medicine. 1998;188(5):985-90.

[20] Fu SM, Dai C, Zhao Z, Gaskin F. Anti-dsDNA Antibodies are one of the many autoantibodies in systemic lupus erythematosus. F1000Research. 2015;4(F1000 Faculty Rev).

[21] Okamura M, Kanayama Y, Amastu K, Negoro N, Kohda S, Takeda T, et al. Significance of enzyme linked immunosorbent assay (ELISA) for antibodies to double stranded and single stranded DNA in

.

patients with lupus nephritis: correlation with severity of renal histology. Annals of the Rheumatic Diseases. 1993;52(1):14-20.

[22] MacGowan JR, Ellis S, Griffiths M, Isenberg DA. Retrospective analysis of outcome in a cohort of patients with lupus nephritis treated between 1977 and 1999. Rheumatology (Oxford, England). 2002;41(9):981-7.

[23] López-Hoyos M, Cabeza R, Martínez-Taboada VM, Crespo J, SanSegundo D, Blanco R, et al. Clinical disease activity and titers of anti-dsDNA antibodies measured by an automated immunofluorescence assay in patients with systemic lupus erythematosus. Lupus. 2005;14(7):505-9.

[24] Sturfelt G, Truedsson L. Complement in the immunopathogenesis of rheumatic disease. Nature reviews Rheumatology. 2012;8(8):458-68.

[25] Nguyen CP, Cao VV, Feher J, Gergely P. Correlation between the levels of antinuclear antibodies, anti-DNA antibodies, and complement in systemic lupus erythematosus. Acta medica Hungarica. 1988;45(2):145-59.

[26] Narayanan K, Marwaha V, Shanmuganandan K, Shankar S. Correlation between Systemic Lupus Erythematosus Disease Activity Index, C3, C4 and Anti-dsDNA Antibodies. Medical Journal, Armed Forces India. 2010;66(2):102-7.

[27] Einav S, Pozdnyakova OO, Ma M, Carroll MC. Complement C4 is protective for lupus disease independent of C3. Journal of immunology (Baltimore, Md : 1950). 2002;168(3):1036-41.

[28] Walport MJ. Complement and systemic lupus erythematosus. Arthritis research. 2002;4 Suppl 3:S279-93.

[29] Moura CG, Lima I, Barbosa L, Athanazio D, Reis E, Reis M, et al. Anti-C1q antibodies: association with nephritis and disease activity in systemic lupus erythematosus. Journal of Clinical Laboratory Analysis. 2009;23(1):19-23.

[30] Lood C, Gullstrand B, Truedsson L, Olin AI, Alm GV, Rönnblom L, et al. C1q inhibits immune complex–induced interferon-α production in plasmacytoid dendritic cells: A novel link between C1q deficiency and systemic lupus erythematosus pathogenesis. Arthritis & Rheumatism. 2009;60(10):3081-90.

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## Declarations

### Authors' contributions

All of authors read and approved the final manuscript.

### Ethics approval and consent to participate

Approved by the Ethic Review Committee (Approval number 312/Panke.KKE/I/2013)

### Consent for publication

Applicable

### Availability of data and materials

Yes

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## Competing interests

The authors declare that they have no competing interests


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