國立臺灣大學醫學院臨床醫學研究所

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Master Thesis

血清鐵蛋白與C反應蛋白於全身性紅斑性狼瘡患者感染時之角色

The Role of Serum Ferritin and C-Reactive Protein in Systemic Lupus Erythematosus Patients with Infection

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口試委員會審定書
MASTER'S THESIS ACCEPTANCE CERTIFICATE
NATIONAL TAIWAN UNIVERSITY

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感染時之角色

The Role of Serum Ferritin and C-Reactive Protein in Systemic Lupus Erythematosus Patients with Infection

本論文係 陳仁豪 (姓名) P08421307 (學號) 在國立臺灣大學 臨床醫學研究所 完成之碩士學位論文，於民國 112 年 7 月 12 日承下列考試委員審查通過及口試及格，特此證明。

The undersigned, appointed by the Institute of Clinical Medicine on 12 July, 2023 have examined a Master's thesis entitled above presented by JEN-HAO CHEN (P08421307) candidate and hereby certify that it is worthy of acceptance.

口試委員 Oral examination committee:

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誌 謝

感謝賢內助劭青在繁忙的工作仍一肩扛起家裡大小事，讓我能沒有後顧之憂完成課業，感謝謝松洲老師在每個困難時刻陪我討論解決問題，很幸運能在老師的實驗室學習，可以跟著個性沉著和實驗技術經驗一流的劉志仁學長、陳佳玲學姊一步一步完成實驗是我生命中難忘的回憶。很榮幸可以獲得蔡長祐教授與黃妙慈副教授兩位老師的口試與論文指導，謝謝您們鉅細靡遺地提點論文重點，更幫我逐字批改文法、教導寫作方式，協助我大幅提升這份論文的品質，謝謝您們。

我的實驗目標是如何用ELISA法檢測血清Glycosylated Ferritin濃度，以作為紅斑性狼瘡患者疾病發炎與細胞破壞之指標。在這邊學習的第一件事是如何在實驗過程中保護自己與同仁，第二件事是在正式實驗前，將每個步驟細節寫下來，預先演練每一步驟，將會用到的儀器、試劑、環境設定都準備。第三件事是確保檢體運送與保存穩定。很感謝實驗室大家庭的包容與鼓勵，尤其是劉大哥，面對我許多基礎到不行的問題，都能不厭其煩地耐心回覆，陪我一次次演練實驗流程，每次趕到實驗室都已是他的下班時間，但他從不會因此少花一點時間訓練、幫助我。期許自己接下來能再將實驗技巧與檢體保存方式精進，能在下次分析中得到更正確的實驗結果，也將引入Heavy/Light chain Ferritin and NF-κB protein的分析。最後要特別感謝周祖述所長的教導，接下來會想辦法學習如何做全血檢體蒐集並純化mitochondria萃取出NF-κB protein的分析。

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中文摘要

背景：

全身性紅斑性狼瘡患者死亡率最高之原因為感染(31.1%)。然而在免疫功能低之狀況下，患者臨床許多感染症狀，例如發燒、心跳加快等...不一定會出現，又實驗室的檢查，包括白血球增多症、急性反應蛋白(紅血球沉降速率、 C反應蛋白、前降鈣素...)上升，亦時常無法早期觀察到，因而造成診斷與治療上之延遲，影響到病人存活率。過去研究發現C反應蛋白似乎可以做為全身性紅斑性狼瘡患者早期感染指標，惟僅限於非活動期的狼瘡患者。因此，如何找到合適的指標來提早診斷活動性狼瘡患者的感染事件是臨床醫師極大的挑戰。本論文的實驗目標係如何尋找、分析、驗證可靠的實驗室檢驗指標，以期能達到早期診斷紅斑性狼瘡患者感染症的目的。

實驗方法:

實驗期間為民國109年1月1日至民國112年1月31日，我們蒐集21位免疫疾病患者之資料(紅斑性狼瘡13位，其他免疫疾病8位)，其他免疫疾病包含ANCA血管炎3位、原發性乾燥症2位、多發性肌炎2位、全身性硬化症1位；共記錄到25次的感染事件(紅斑性狼瘡15次，其他免疫疾病8次；非典型感染12次，非典型感染13次)。收治醫院為台大醫院與台大雲林分院。急性期檢體的採檢，在首次診斷感染七日內進行。檢測的發炎指標包含血清鐵蛋白[ferritin]與C反應蛋白[CRP]。紅斑性狼瘡患者同時也會使用紅斑性狼瘡活性指標評估，(SLEDAI 2K)，指數≥7.4代表高活性)。基礎期檢體採檢於疾病恢復期，或採用感染前12周內之檢體，檢測血清鐵蛋白及C反應蛋白。統計分析方式採「無母數配對檢定-魏克生符號檢定」(Wilcoxon signed-rank test)，分析感染前後鐵蛋白與C反應蛋白之變化。對於高度懷疑感染的紅斑性狼瘡患者，我們分析「接收者操作特徵曲線」(receiver operating characteristic curve，又稱ROC曲線)，以計算出判斷感染的臨界值。

為分析高鐵蛋白血症(hyperferritinemia)之成因，我們使用酵素結合免疫吸附分析法(enzyme-linked immunosorbent assay, ELISA)檢測血清醣化鐵蛋白分率(Glycosylated Ferritin (GF)/Ferritin ratio)，正常人分率為50%至80%。若結果明顯高

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於50%，傾向細胞受損釋放出其內醣化鐵蛋白引起；若結果明顯低於50%，則傾向活化發炎體(inflammasome)路徑引起。

結果：

第一組：活動期紅斑性狼瘡非典型感染(n=6平均活性指標SLEDAI:14.7，屬高活性)，基礎平均血清球蛋白值為429ng/mL，感染平均血清球蛋白值為2919ng/mL (*p*=0.0277)。基礎平均血清C反應蛋白值為0.32mg/dL，感染平均血清C反應蛋白2.68mg/dL(*p*=0.0277)。

第二組：活動期紅斑性狼瘡細菌感染(n=9平均活性指標SLEDAI:11.1 高活性)，基礎平均血清球蛋白值為157ng/mL，感染平均血清球蛋白值為1588ng/mL (*p*=0.0077)。 基礎平均血清C反應蛋白值為0.18mg/dL，感染平均血清C反應蛋白9.7mg/dL(*p*=0.0076)。

第三組:其他免疫疾病非典型感染(n=7)
基礎平均血清球蛋白值為293ng/mL，感染平均血清球蛋白值為1130ng/mL (p=0.018). 基礎平均血清C反應蛋白值為0.45mg/dL，感染平均血清C反應蛋白3.28mg/dL(p=0.028)。

第四組: 其他免疫疾病細菌感染(n=3)
基礎平均血清球蛋白值為462ng/mL，感染平均血清球蛋白值為10768ng/mL (p=0.285). 基礎平均血清C反應蛋白值為0.45mg/dL，感染平均血清C反應蛋白15.1mg/dL(p=0.108)。

自體免疫疾病(包含紅斑性狼瘡與其他免疫疾病)預測感染之ROC曲線分析最佳預測值位於400-799 ng/mL區段，其平均值為600ng/mL (敏感度:73% 特異性:95.23%) 。

為分析同一位活動期紅斑性狼瘡患者遭遇細菌與非典型感染時，C反應蛋白與球蛋白上升的情況，我們分析四位病患，使用C反應蛋白差值(ΔCRP: 感染期-基礎

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期)、血清鐵蛋白差值(ΔFerritin: 感染期-基礎期)、C反應蛋白差值與血清鐵蛋白差值比值(ΔFerritin/ΔCRP)作為分析指標。

結果顯示兩者ΔCRP皆有顯著統計學差異。平均ΔCRP在非典型感染為2.87mg/dL，在細菌感染為14.89mg/dL, p=0.01738，而Δferritin及ΔFerritin/ΔCRP則明顯無差異，表示活動性紅斑性狼瘡患者C反應蛋白於非典型感染時相較細菌感染血清鐵蛋白無法顯著上升，難以預測感染症;而血清鐵蛋白不論是在非典型或細菌感染皆有顯著差異。

血清醣化鐵蛋白分率結果分為兩群：活動性紅斑性狼瘡腎炎患者遭遇細菌或隱球菌感染時，其平均分率為17.35% (0.54 - 32%；n=4)；以及活動性紅斑性狼瘡腎炎患者且無感染者，其平均分率為39.67% (9.9 - 57%；n=3)。此結果顯示活動性紅斑性狼瘡遭遇細菌或隱球菌感染時，血清醣化鐵蛋白分率偏低， 推測為其高鐵蛋白血症較傾向為活化發炎體(inflammasome)路徑之結果。

結論：血清鐵蛋白≥600ng/mL可作為免疫疾病(包含全身性紅斑性狼瘡與其他免疫疾病)之早期感染偵測指標。血清鐵蛋白在活動性紅斑性狼瘡病人感染時仍能穩定升高，不論是對細菌感染或是非典型感染都有極佳的預測性。反觀C反應蛋白，在活動性狼瘡的病人遭遇非典型感染時預測性不佳，在非活動性紅斑性狼瘡或是細菌感染的情況較能明顯上升。而活動性紅斑性狼瘡患者遭遇感染引發之高鐵蛋白血症，傾向是因活化發炎體(inflammasome)路徑所引起。故臨床上當我們難以區分紅斑性狼瘡患者為感染或是疾病活性上升時，同時檢測血清鐵蛋白與C反應蛋白可以幫助我們更精確判斷為何者。

#紅斑性狼瘡Systemic lupus erythematosus #鐵蛋白Ferritin #C反應蛋白 CRP #感染Infection #非典型感染Atypical infection

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英文摘要

Background:

The main cause of mortality in SLE patient is infection (31.1%). Patients with immunocompromised conditions often do not show common signs of infection, such as fever, tachycardia, leukocytosis, and acute phase protein elevation (CRP, erythrocyte sedimentation rate (ESR), procalcitonin [PCT]). In previous studies, CRP (≥60.0mg/L) seems to be a valuable marker for diagnosing infection; however, it is only limited to inactive SLE patients. Therefore, how to recognize infection in active SLE patients imposes a difficult challenge. The aim of our study is to find a reliable laboratory marker for early diagnosis of infection in SLE patients.

Methods:

Between January 1, 2020 to January, 31, 2023, we gathered data from 21 patients (SLE: n=13, other autoimmune diseases n=8) from NTUH and NTUH-Yunlin and recorded a total of 25 infection incidences (SLE: n=15; Other: n=10). We analyzed the SLE patients' disease activity, as measured by the SLEDAI 2k score, as well as their inflammation markers (serum ferritin and CRP), within 7 days of their infection or within 12 weeks before the infection and at the time of recovery. The Wilcoxon signed-rank test was used to compare the baseline data. To determine the cut-off level of serum ferritin in SLE patients highly suspected to have infection, we used the Receiver Operating Characteristic (ROC) curve.

To clarify the etiology of hyperferritinemia, we analyzed serum glycosylated ferritin/ferritin ratio using an enzyme-linked immunosorbent assay (ELISA) kit.

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If glycosylated ferritin (GF)/ferritin ratio is low, lupus-related reaction is likely. On the other hand, if glycosylated ferritin/ferritin ratio is high, this could mean cell damage and glycosylated ferritin release from the lysed cells.

Result:

Group 1 Active SLE with atypical infection (SLEDAI:14.7 High activity):

The mean serum ferritin levels were 429ng/mL (baseline) and 2919 ng/mL (infection)(p=0.0277). The mean CRP levels were 0.32mg/dL (baseline) and 2.68mg/dL (infection)(p=0.0277).

Group 2 Active SLE with bacterial infection (SLEDAI:11.1, High activity):

The mean serum ferritin levels were 157ng/mL (baseline) and 1588ng/mL (infection)(p=0.0077). The mean CRP levels were 0.18mg/dL (baseline) and 9.7mg/dL (infection)(p=0.0076).

Group 3 Other autoimmune disease with atypical infection (n=7):

The mean serum ferritin levels were 293ng/mL (baseline) and 1130ng/mL (infection)(p=0.018). The mean CRP levels were 0.45mg/dL (baseline) and 3.28mg/dL (infection)(p=0.028).

Group 4 Other autoimmune disease with bacterial infection (n=3):

The mean serum ferritin levels were 462ng/mL (baseline) and 10768ng/mL (infection)(p=0.285). The mean CRP levels were 0.45mg/dL (baseline) and 15.10mg/dL (infection)(p=0.108).

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The cut-off value of ferritin level for infection in patients with autoimmune diseases (SLE or others) was 600ng/mL (400-799) (sensitivity: 73% specificity:95.23) as calculated by using the Receiver Operating Characteristic (ROC) curve.

The mean ΔCRP(infection-baseline) of the same patient with atypical and bacterial dual infections were(n=4) atypical: 2.87 mg/dL, bacterial:14.89 mg/dL and with a statistically significant difference (p=0.01738). However, the mean ΔFerritin or ΔFerritin/ΔCRP ratio did not show significant difference.

The GF/Ferritin ratios were lower in the SLE with infection group than the disease flare group (mean 17.35%, 0.54 – 32.7% *vs* 39.67%, 9.9 – 57%), which indicated that the result of hyperferritinemia in active SLE patients with infection may be due to overexpression of the inflammasome pathway rather than cell damage/lysis.

Conclusion:

Serum ferritin increases steadily in patients with active lupus erythematosus contracted with bacterial or atypical infections. Serum ferritin level with a cut-off value of ≥ 600ng/mL can be used as an early infection indicator in patients with autoimmune diseases, including systemic lupus erythematosus and other autoimmune diseases. In contrast, CRP is not predictive of atypical infections in patients with active lupus, but can be seen to increase significantly in patients with inactive lupus erythematosus with bacterial infection. Patients with active lupus erythematosus encounter infection-induced hyperferritinemia, which tends to be caused by activation of the inflammasome pathway.

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Therefore, clinically when it is difficult to differentiate between SLE patients with infections or disease flare, testing both ferritin and CRP can assist practitioners make a more precise diagnosis.

#Systemic lupus erythematosus #Ferritin #C-reactive protein #Infection #Atypical infection

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碩士論文內容

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緒論:

Background for Systemic Lupus Erythematosus (SLE)

SLE is a severe autoimmune disease which could result in life-threatening conditions. The prevalence is about 73 out of 100,000 globally, with a female predominance (female: male= 9:1). However, male has higher risks of poor outcome. The reason for female predominance may be due to predisposing gene variants located in the X chromosome (IRAK1, MECP2, TLR7) [1](Rullo, O.J., et al. 2013). A majority of patients' disease onset age range between 16 and 55 (consisting of 65% of the patients). The poor prognosis factor includes male, young age, and patients of African American or Mexican Hispanic descent.

The pathogenesis of SLE is multifactorial (epigenetic, genetic, ecological, hormonal and environmental factors). Patients with susceptibility genes, such as HLA-DR2/3, GLK, PTPN22, TLR-7, and TLR-9 and etc. are exposed to trigger factors (ex: UV B, infection and etc.) that generate abnormal innate and adaptive immune responses. Inadequate clearance of immune complex results in organ inflammation and damage [2] (Ameer, M.A., et al.2022).

Genetic factors

Monogenic disease of SLE is rare but with highest hazard ratios (HR) of 5 to 25. Heterozygous mutation in the TREX1 gene and complement C1q deficiencies had been reported [3] (Demirkaya, E., et al., 2020).

SLE patient often has active type 1 interferon expression (IFN-alpha, innate

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immunity). Susceptibility loci genes, IRF5, STAT4, IRAK1, TNFAIP3, SPP1 and TLR7/9, are associated with IFN-alpha pathways [4](Bronson, P.G., et al., 2012).

Environmental factors

The environmental factors associated with SLE includes UV B, infection, silicon, smoking, air pollution and heavy metals. UV light may cause T cell auto-reactivity by overexpression of lymphocyte function-associated antigen (LFA)-1 [5](Yung, R., et al., 1996). For infection, Mycobacterial or Epstein-Barr virus (EBV) may result in elevation of anti-DNA antibodies and trigger SLE onset [6] (Steinberg, A.D., et al, 1998). Silica dust could induce SLE [7] (Cooper, G.S., et al., 1998).

Hormonal factors

Given the fact that SLE occurs more often in the female, it has been demonstrated that estrogen is causally related to pathogenesis of SLE). In this aspect, studies have shown that estrogen is able to stimulate the type 1 IFN pathway, which in turn induces SLE activity [7] (Cooper, G.S., et al., 1998). Although studies have proven interconnectivity between multiple factors, the exact etiology of SLE remains unknown (*see* **Figure 1**).

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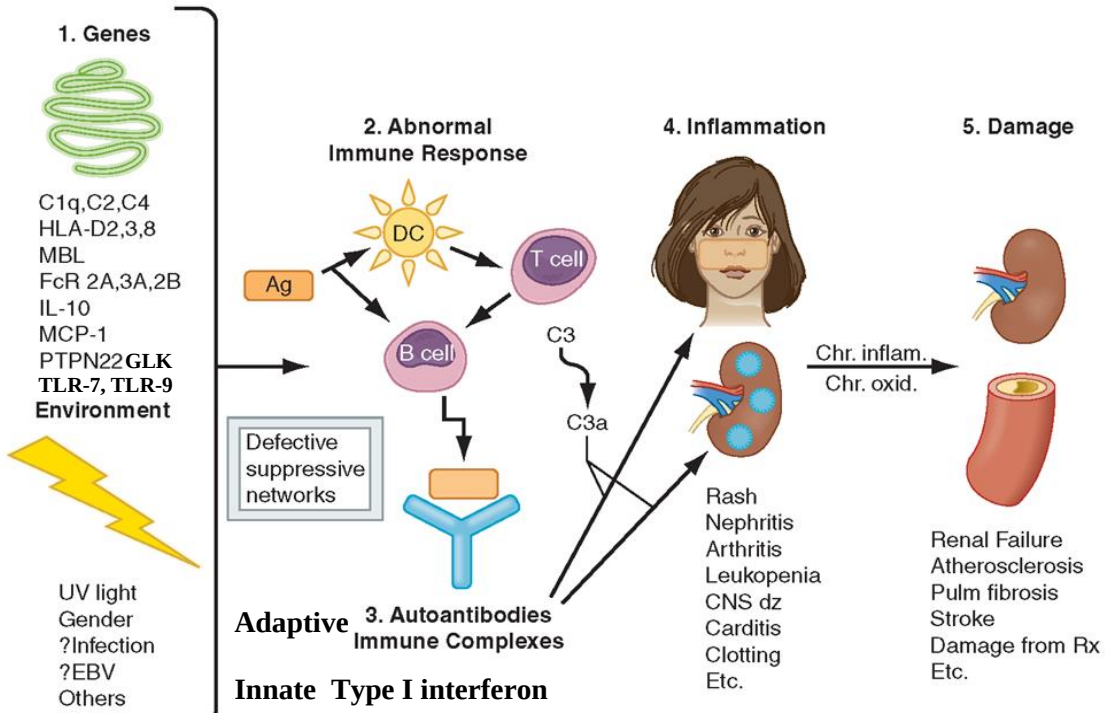

Adapted from Harrison Principle Internal Medicine 18e

Figure 1. Etiology of SLE

Genetically predisposed patients develop SLE upon encounters with trigger factors including epigenetic, ecological, hormonal, and environmental factors . Abnormal immune response includes adaptive immunity (T cell and B cell autoreactivity, immune complexes) and innate immunity (interferon-alpha, damage, macrophage function).

Causes of mortality in SLE Patient

The leading causes of mortality in SLE patients are infections (31.1%) and renal diseases [8, 9] (Moghaddam, B., et al. 2021; Mu, L., et al. 2018). However, there is currently no trustworthy model for early detection of infection in active SLE patients. Because patients with immunocompromised conditions often do not show common signs of infection, such as fever, tachycardia, leukocytosis, and acute phase protein elevation (CRP, erythrocyte sedimentation rate (ESR), procalcitonin (PCT)), it is imperative to establish a model for early detection of infection in active SLE patients.

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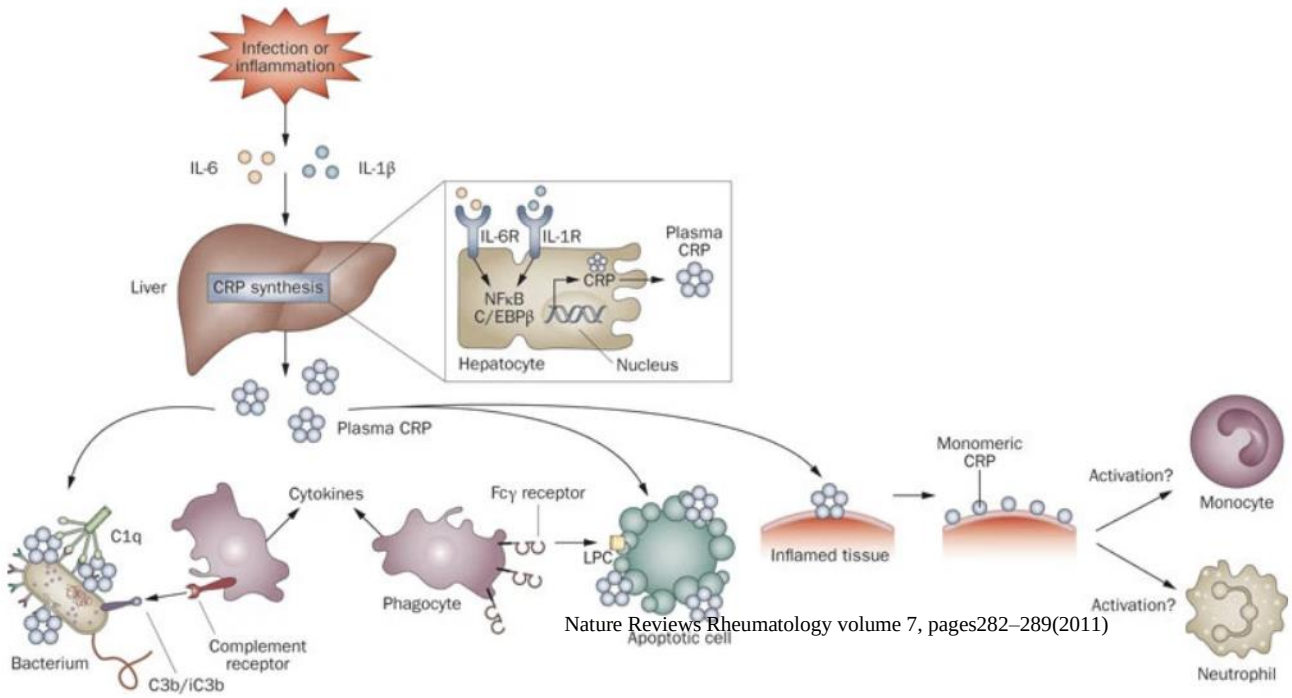
C-reactive protein (CRP)

C-reactive protein (CRP) is a pentameric protein. Native CRP (nCRP) is synthesized by liver hepatocytes primarily. Other sites of CRP production include smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes. At the infection/inflammation site, CRP increases up to 1,000-fold and dissociates into five separate monomers, also known as monomeric CRP (mCRP) [10] (Sproston, N.R. and J.J. Ashworth, 2018)(*see* **Figure 2**).



Nature Reviews Rheumatology volume 7, pages282–289(2011)

Figure 2. CRP Synthesis Pathway

Infection or inflammation triggers IL-6 to induce CRP production in liver via the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) protein.

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Inflammation or infection induces pro-inflammatory cytokine IL-6 production which stimulates CRP synthesis. CRP opsonizes pathogens, decomposes host-debris, and aids phagocytosis. CRP activates complement pathways, apoptosis, phagocytosis, nitric oxide (NO) release, and the production of cytokines, particularly IL-6 (positive feedback) and tumor necrosis factor-α.

Upon encountering the incoming pathogens, CRP stimulates the C1q molecule of the complement pathway to opsonize the pathogens. The classical complement initiatorC1q and the inhibitor C4bp compete for mCRP binding, controlling the local balance of mCRP in tissues [11] (Mihlan, M., et al., 2011).

On the other hand, CRP can also launch cell-mediated pathways by activating complement binding to Fc receptors of IgG, which leads to the release of pro-inflammatory cytokines [12](Du Clos, T.W., 2000).

Furthermore, the apoptosis process could also be triggered by CRP [13] (Devaraj, S., T.W. Du Clos, and I. Jialal, 2005). CRP stimulates the production of pro-apoptotic cytokines IL-1β, tumor necrosis factor-α (TNFα), reactive oxygen species (ROS)), and inflammatory mediators via the activation of Fcγ receptors [14](Kobayashi, S., et al., 2003).

CRP plays an important role in infection and immunity. However, there is no association between the infection types and CRP levels [15] (Healy, B. and A. Freedman, 2006).

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In cardiovasculardiseases, especially in asymptomatic individuals, elevated serum CRP levels is a strong independent predictor for cardiovascular diseases such as atherosclerosis, congestive heart failure, atrial fibrillation, myocarditis, and aortic valve disease [16] (Ridker, P.M., et al., 2002).

High-sensitivity CRP assays are used to detect baseline levels of CRP in patients at risk of cardiovascular diseases. Serum CRP level higher than 3 mg/L raises the risk of coronary heart disease and type 2 diabetes [17, 18](Kushner, I., 1990; Soinio, M., et al., 2006).

Hormone replacement therapy (HRT) could also influence CRP levels. One nested case-control study of post-menopausal women in the United States showed that HRT in post-menopausal women caused increased levels of serum CRP. However, HRT itself had less correlation with cardiovascular risk than the serum CRP or IL-6 level [19](Pradhan, A.D., et al., 2002).

In summary, CRP is widely used in detecting an infection or /inflammation event clinically.[10]. However, CRP itself is not specific for any definite pathogen or immune pathway.

Erythrocyte sedimentation rate (ESR)

ESR measures the descending rate of the red blood cells in anticoagulated whole blood of the standardized tube over one hour. It is non-specific for any single disease. ESR is influenced by the degree of aggregation of the red blood cells. Blood plasma proteins, fibrinogen, would hasten the formation of red cell clusters (rouleaux).

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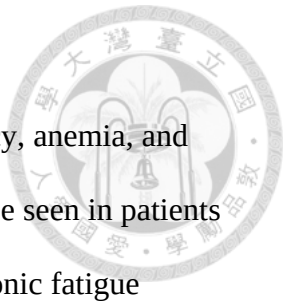

High ESR could be a result of infection, inflammation, pregnancy, anemia, and cancers such as lymphoma and multiple myeloma. Low ESR could be seen in patients with polycythemia, hyperviscosity, sickle cell anemia, leukemia, chronic fatigue syndrome, low plasma protein due to liver or kidney disease and congestive heart failure [20] (Brenu, E.W., et al., 2010). ESR as an inflammatory marker widely used to actively monitor infectious or inflammatory diseases. The normal range of ESR (mm/h) is under [Age(in years) + 10(if female)]/2.

Procalcitonin

Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin. It is produced by the parafollicular cells, also called C cells, of the thyroid gland and by the neuroendocrine cells of the lung and the intestine. The normal serum level of PCT in healthy individuals is below 0.01 μg/L. Infection, particularly bacterial infection, stimulates PCT production [21](Reinhart, K., W. Karzai, and M. Meisner, 2000). The induction period for PCT ranges from 4to 12 hours with a half-life spanning anywhere from 22 to 35 hours [21](Reinhart, K., W. Karzai, and M. Meisner, 2000). It does not increase significantly with viral or non-infectious inflammations [22](Jin, M. and A.I. Khan, 2010).

In systemic inflammatory response syndrome (SIRS), PCT has a sensitivity of 90% and a specificity of 91% compared with IL-2, IL-6, IL-8, CRP and TNF-alpha.[23](Balc, I.C., et al., 2003). It is a useful tool in guiding the initiation and duration of antibiotic treatments in patients with bacterial pneumonia and other acute respiratory infections (The Cochrane Database of Systematic Reviews 10(5):

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CD007498.) However, in SLE patients, PCT has a low prediction rate for infection. In contrast, PCT has been shown a positive correlation to SLE activity[24] (Song, G.G., S.C. Bae, and Y.H. Lee et al. 2015).

Studies on predicting infection event in SLE patients

In previous studies, CRP (≥60mg/L) seems to be a valuable marker for recognizing infection [25](Cengić, M., et al. 2002); however, they are only limited to inactive SLE patients [24, 26](Wang, J., et al. 2019; Song, G.G., S.C. Bae, and Y.H. Lee et al. 2015).

The reason for limited elevation of CRP in active SLE patients may be due to Interferon Gene Signature (IGS) and CRP-lowering polymorphism, *rs*1205, overrepresentiveness [27] (Enocsson, H., et al. 2021). Therefore, how to recognize infection early on in active SLE patients imposes a difficult challenge. The aim of our study is to find an available laboratory marker for early diagnosis of infection in SLE patients.

Introduction for Ferritin

Ferritin as an iron-binding protein also plays a role in inflammation (light chain) and immunomodulation (heavy chain). Ferritin is a soluble 450 kilo-Dalton (kDa) protein with 24 apoferritin monomers. It exists in all cells, especially in marrow macrophages, spleen, and liver [28] (Cullis, J.O., et al. 2018).

About 50–80% of serum ferritin is glycosylated in the reticuloendothelial system (RES). Glycosylated ferritin has a longer half-life of about 50 hours than the non-glycosylated form of 5 hours). The normal range of serum ferritin is 40-200 ng/mL. As

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an acute phase protein, serum ferritin elevates in 6 hours after trauma, and is degraded at day 7 [29](Northrop-Clewes, C.A., et al. 2008).

Studies for ferritin in SLE patient

The baseline of serum ferritin level in SLE patient is higher than healthy people (SLE mean: 245.3 ng/ml; Healthy controls mean: 23.11 ng/ml))[30] (Tripathy, R., A.K. Panda, and B.K. Das., et al. 2015). The level of serum ferritin correlates with disease activity and renal dysfunction in SLE.

Differential diagnosis of hyperferritinemia

The differential diagnosis of hyperferritinemia includes iron overload, infection, malignance, cellular damage, chronic liver disease, and autoimmune disease [31](Sandnes, M., et al., 2021).

Blood transfusion and hyperferritinemia

Recent blood transfusion within 14 days has limited influence on serum ferritin levels [32, 33] (Ho, C.H., 1992; Berz, D., et al. 2006). However, repeated blood transfusions, over eight units of RBC for one year, could result in hyperferritinemia.

Role of hyperferritinemia in active SLE patients with infection

According to our clinical experience, SLE patients suffering from infection have higher levels of serum ferritin. Hyperferritinemia may be the result of cell damage or lupus-related reaction. Hyperferritinemia in SLE patients may be caused by type 1 interferon (IFN) regulated genes. Interferon gene signature (IGS) over-activates toll-like receptor-9 (TLR-9) expression, which stimulates IL-18 secretion and causes activates

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inflammasome pathway, leading to hyperferritinemia [34] (Girard-Guyonvarc'h, C., et al., 2018)

NF-κB is essential for both CRP production and inflammasome activation. We hence hypothesize that overactive inflammasome activation in SLE patients competitively usurps NF-κB and hinders CRP production, resulting in low CRP production of these patients as clinically observed. (*see* **Figure 2, 3**).

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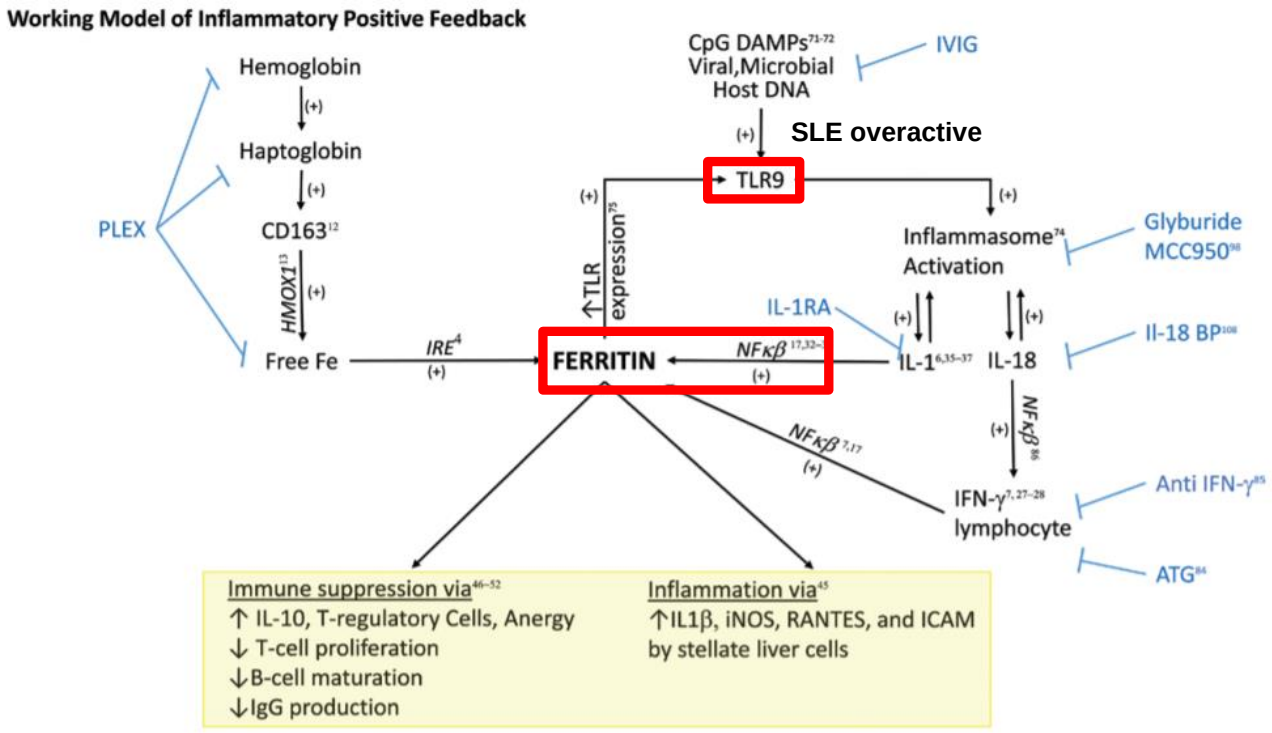

Adapted from International Immunology, Vol. 29, No. 9, pp. 401–409(2017)

Figure 3. Ferritin and inflammasome

In both normal and SLE patients with viral infection, inflammasome pathway is initiated by TL-9. TLR-9 stimulates the releases of IL-1 and IL-18 cytokines, leading to ferritin secretion via NF-κB activation. However, compared to normal patients, SLE patients with TLR-9 mutation will initiate overactive inflammatory positive feedback pathways, leading to feed-forward inflammasome and NF-κB activation and ferritin overproduction.

To clarify the etiology of hyperferritinemia, we analyzed serum glycosylated ferritin/ferritin ratio using an enzyme-linked immunosorbent assay (ELISA) kit.

If glycosylated ferritin/ferritin ratio is low, lupus-related reaction is likely. On the other hand, if glycosylated ferritin/ferritin ratio is high, this could mean cell damage

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and glycosylated ferritin release from lysed cells.

Based on the above information, we hypothesize that serum ferritin may be an effective biomarker to early detect infection events in active SLE patients.

Aims of the study

1. Find a reliable laboratory test for early detection of infection event in active SLE patients. Compare ferritin and CRP levels at the acute infection stage with the baseline condition.
2. Explain the etiology of hyperferritinemia development and analyze glycosylated ferritin/ferritin ratio by ELISA kit.

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Patient recruitment and evaluation

We recruited patients with SLE or other autoimmune diseases from National Taiwan University Hospital (NTUH) and NTUH-Yunlin who were diagnosed to have infections between January 1, 2020 to January 31, 2023. The acute phase reactant blood tests were performed within 7 days after the infection event; baseline blood tests were performed after the infectious disease subsided. If a blood test could not be carried out, a previous data without acute infection within 12 weeks was taken as the baseline data.

The SLE was diagnosed according to the SLICC (Systemic Lupus International Collaborating Clinics Classification Criteria for Systemic Lupus Erythematosus) 2012 criteria. The procedures were undertaken after an IRB (Institutional Review Board) certificate and informed consents were obtained.

The SLICC 2012 criteria is a practical tool for clinical diagnosis of SLE (sensitivity: 97%, specificity: 84%) (Appendix 1)

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Inclusion and Exclusion criteria

Inclusion criteria:

1. Age ≥ 20-year-old
2. Definite autoimmune disease
3. Definite infection event

Exclusion criteria:

1. Age < 20-year-old
2. Hyperferritinemia syndrome (septic shock, hemophagocytic lymphohistiocytosis (HLH)), Still's disease, and catastrophic antiphospholipid syndrome)
3. Iron overload (serum ferritin≥1000 without infection)
4. Blood test over 7 days after infection event
5. Pregnancy
6. Malignancy

Patients were divided into four groups:

Group 1: Active SLE with atypical infection;

Group 2: Active SLE with bacterial infection;

Group 3: Other autoimmune disease with atypical infection;

Group 4: Other autoimmune disease with bacterial infection.

Disease activity evaluation

We analyzed the SLE patients' disease activity by SLEDAI 2k score (Appendix 2、3) [14] (Gladman, D.D., D. Ibañez, and M.B. Urowitz., et al. 2002). A mean score of ≥7.4 indicates high disease activity. Blood test of inflammation markers including

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serum ferritin and CRP were performed within 7 days after infectious events developed.

Statistical methods

The Wilcoxon signed-rank test was used to compare data in the infection phase and the recovery phase. Receiver Operating Characteristic (ROC) was used for cut-off level evaluation of definite infection.

Glycosylated Ferritin analysis

The serum glycosylated ferritin was measured by *Human Glycosylated Ferritin competitive ELISA kit (*BT LAB) (Appendix 4). We collected patients' serum samples and kept them at -80℃. Before running the assay, we thawed the samples at room temperature for 30 minutes and prepared all the reagents with distilled water. The reagents include standards, biotinylated antigen, avidin- horseradish peroxidase (HRP), substrate solution A/B, stop solution, and wash buffer. Then we prepared standards (150µl in 6ml) with serial dilutions of 1200ng/ml, 600ng/ml, 300ng/ml, 150ng/ml and 75ng/ml.

At the start of ELISA, we added 50µl samples and standards to the plate pre-coated with anti-glycosylated ferritin antibody. Then, we added 50µl biotinylated antigen to each well and incubated them at 37℃ for 60 minutes. At this time, the antigens in the samples competed with the biotinylated antigen to bind to the capture antibody. Then, we washed five times with 300µl wash buffer to remove any unbound antigen. 50µl avidin-HRP was added afterwards and then incubated for 60 minutes at 37℃. Unbound avidin-HRP was washed away during the washing step. We then added substrate solution A/B (TMB Substrate, 3, 3', 5, 5'-tetramethylbenzidine) and incubated it at 37℃

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for 25 minutes in the dark. After colors developed, we added 50μl of stop solution (acidic stop solution) to each well and colors changed into yellow.

As we put the plate in microplate spectrophotometer (Spectra ABS Plus), glycosylated ferritin (GF) was measured at 450 nm. The intensity of the colors developed was inversely proportional to the concentration of GF in the sample. We then determined the concentration of GF in the sample by comparing the O.D. of the samples to the standard curve. (Charbonnet, Derrick., issued August 3, 2010)

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結果與討論

Patient characteristics

Between January 1, 2020 to January 31, 2023, we gathered data from 21 patients (SLE: n=13, other autoimmune diseases n=8) from National Taiwan University Hospital (NTUH) and NTUH-Yunlin, and recorded a total of 25 infection incidents (SLE: n=15 Other: n=10).

The patient characteristics are listed in Table 1. There was no severe anemia or iron overload. The baseline serum ferritin was higher in Group 1 (SLE and atypical infections) and Group 4 (other autoimmune diseases with bacterial infections) than previous studies. The mean SLEDAI scores of Group 1 and 2 were above ≥7.4, indicating high disease activity.

Table 1. Patient Characteristics

	1. SLE, atypical	**2. SLE, bacteria**	**3. Others, atypical**	**4. Others, bacteria**
Event	n=6	n=9	n=7	n=3
Age, mean ±SD (y/o)	64.2±17.6	56.2±27.8	67.0±15.0	65.3±3.5
Hb, mean ±SD (g/dL)	9.9±2.1	9.9±1.9	11.9±2.4	12.1
Fe, mean ±SD (μg/dL)	81.0±21	71.5±29	52.71±20.6	56.6±16.9
Ferritin, mean ±SD (ng/dL)	429±309	157±105	293.6±330	462.33±508
CRP, mean ±SD (mg/dL)	0.32±0.28	0.19±0.20	0.45±0.33	0.45±0.40
SLEDAI, mean	11.1(8-22)	14.7(0-22)		

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The clinical characteristics of SLE patients were lupus nephritis (n=11), AIHA (autoimmune hemolytic anemia, n=2), APS (antiphospholipid syndrome, n=1), and low complement (n=1).

Other autoimmune diseases included ANCA vasculitis (n=3), Sjögren's syndrome (n=2), polymyositis (n=2), systemic sclerosis (n=1), and antiphospholipid syndrome (n=1).

The bacterial infections (n=12, SLE: 9, others: 3) included *Escherichia coli* (SLE: 5, others: 1), *Pseudomonas* (SLE: 2, others: 2), *Acinetobacter* (SLE: 2, others: 0). The atypical infections (n=13, SLE: 6, others: 7) included *Cryptococcus* (SLE: 4, others: 4), *Cytomegalovirus*, CMV (SLE: 1, others: 2), *Hepatitis B virus*, HBV (SLE: 1, others: 0), *Epstein-Barr virus*, EBV (SLE: 0, others: 1).

Results from SLE patients with atypical and bacterial infection

Group 1 (Active SLE with atypical infection, SLEDAI: 14.7):

The mean serum ferritin levels were 429 ng/mL (baseline) and 2919 ng/mL (infection)(p=0.0277). The mean CRP levels were 0.32mg/dL (baseline) and 2.68mg/dL (infection)(p=0.0277).

Group 2 (Active SLE with bacterial infection, SLEDAI: 11.1):

The mean serum ferritin levels were 157ng/mL (baseline) and 1588ng/mL (infection)(p=0.0077). The mean CRP levels were 0.18mg/dL (baseline) and 9.7mg/dL (infection)(p=0.0076) (**Table 2**).

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Table 2. Active SLE with infection

	Atypical Infection (n=6)	**Bacterial Infection (n=9)**
Ferritin, mean, Base (ng/mL)	429±309((255-1038)	157 ±105(63-352)
Ferritin, mean, Acute (ng/mL)	2919±2548(839-7255)	1588 ± 2031(205-5231)
p value	0.0277	0.0077
CRP, mean, Base (mg/dL)	0.32±0.28(0.08-0.76)	0.18±0.20(0.05-0.37)
CRP, mean, Acute (mg/dL)	2.68± 1.60(0.03-4.5)	9.7± 8.4(0.02-23.4)
p value	0.0277	0.0076
SLEDAI (mean)	14.7	11.1

The result showed that serum ferritin level changes were statistically significant in both atypical (p=0.0077) and bacterial infection (p=0.0277). CRP also disclosed statistical significance on both sides. However, CRP elevation was sensitive to many factors, such as trauma and inflammation. According to previous studies, CRP (≥60mg/L) seems to be a valuable marker for diagnosing infection [4] (Cengić, M., et al. 2002).

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Result of other autoimmune disease with atypical and bacterial infection

Group 3 (other autoimmune disease with atypical infection, n=7):

The mean serum ferritin levels were 293 ng/mL (baseline) and 1130 ng/mL (infection)(p=0.018). The mean CRP levels were 0.45mg/dL (baseline) and 3.28mg/dL (infection)(p=0.028).

Group 4 (other autoimmune disease with bacterial infection, n=3):

The mean serum ferritin levels were 462ng/mL (baseline) and 10768ng/mL (infection)(p=0.285). The mean CRP levels were 0.45mg/dL (baseline) and 15.10mg/dL (infection)(p=0.108) (**Table 3**).

Table 3. Other autoimmune diseases with infection

	Atypical Infection(n=7)	**Bacterial Infection(n=3)**
Ferritin, mean, Base (ng/mL)	293± 330(99-1028)	462 ±508(162-1050)
Ferritin, mean, Acute (ng/mL)	1130 ±1177(165-3616)	10768 ±17407(364-30865)
p value	0.018	0.285
CRP, mean, Base (mg/dL)	0.45 ±0.33(0.02-0.85)	0.45 ±0.40(0.14-0.91)
CRP, mean, Acute (mg/dL)	3.48 ±3.1(0.95-9.96)	15.10 ±12.74(0.42-23)
p value	0.028	0.108

Other autoimmune diseases (ANCA vasculitis (n=3), Sjögren's syndrome (n=2), polymyositis (n=2), systemic sclerosiss (n=1), and antiphospholipid syndrome (n=1)) with atypical infection had significant elevation of ferritin (p=0.018) and CRP (p=0.028). Because the number of cases was limited, the results of bacterial infection were not statistically significant.

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Individual serum ferritin and CRP change in active SLE patients

The changes in levels of individual serum ferritin and CRP in active SLE patients with atypical infection are shown in **Figure 4** and **Figure 5**, respectively.

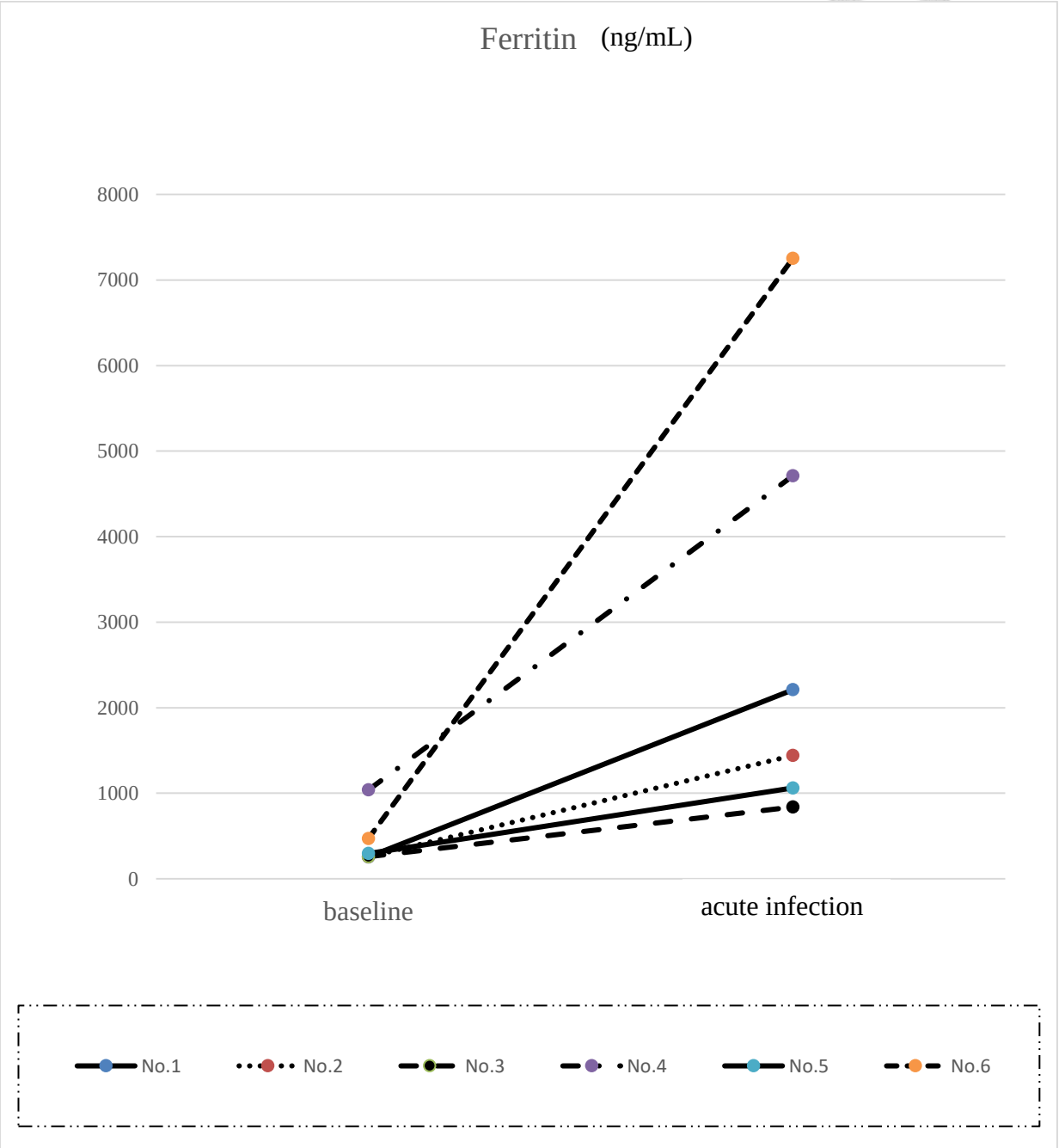

Figure 4. Ferritin change in active SLE patients with atypical infection

In the acute infection phase, active SLE patients displayed significant serum ferritin elevation. The mean serum ferritin levels were over 1000ng/dL in acute

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infection.

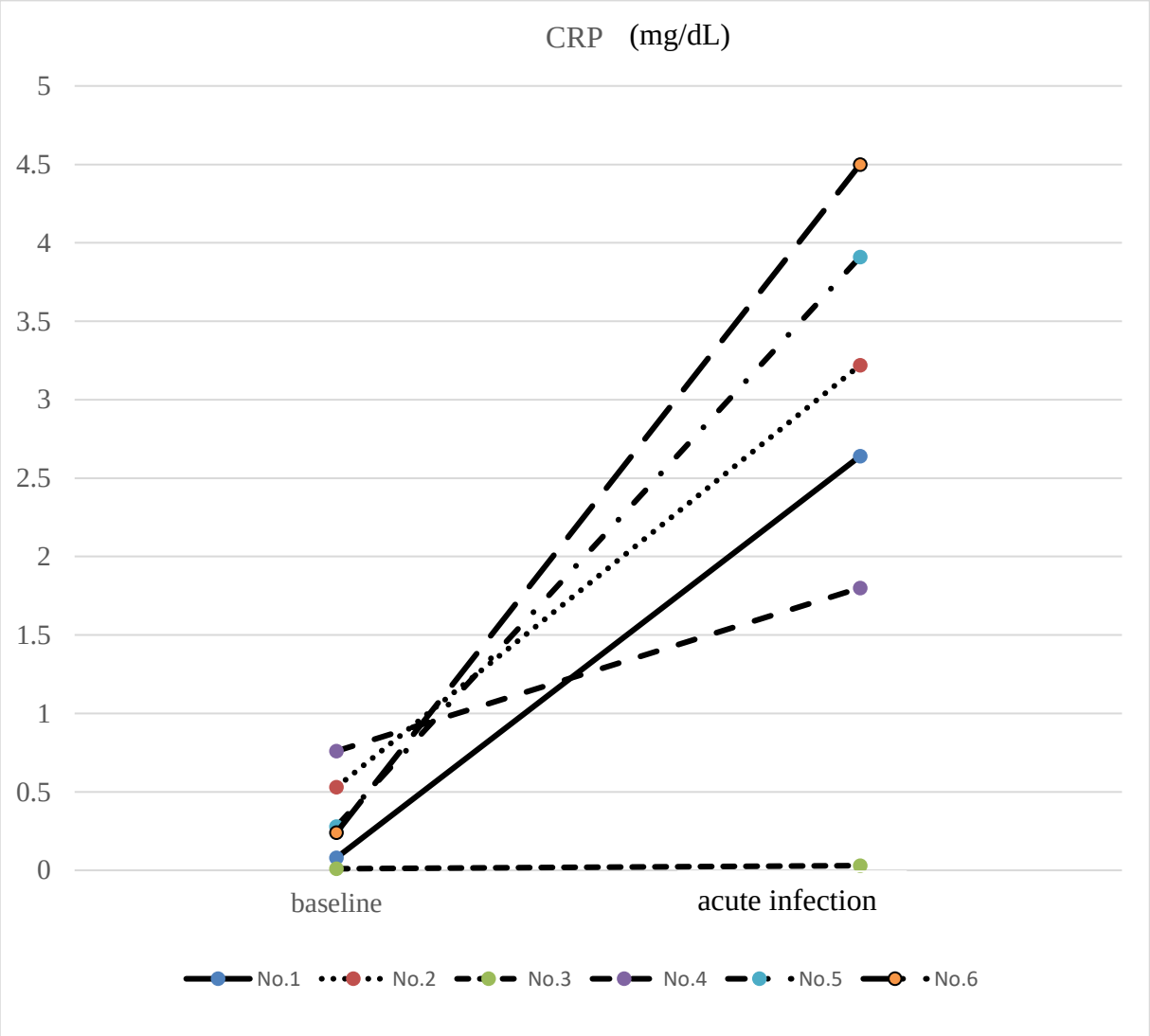

Figure 5. CRP change in active SLE patients with atypical infection

During the acute infection phase, the serum CRP levels of active SLE patients were all under 6mg/dL, with half of them were under 3 mg/dL.

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The change in levels of individual serum ferritin and CRP in active SLE patients with bacterial infection were showed in **Figure 6** and **Figure 7**, respectively.

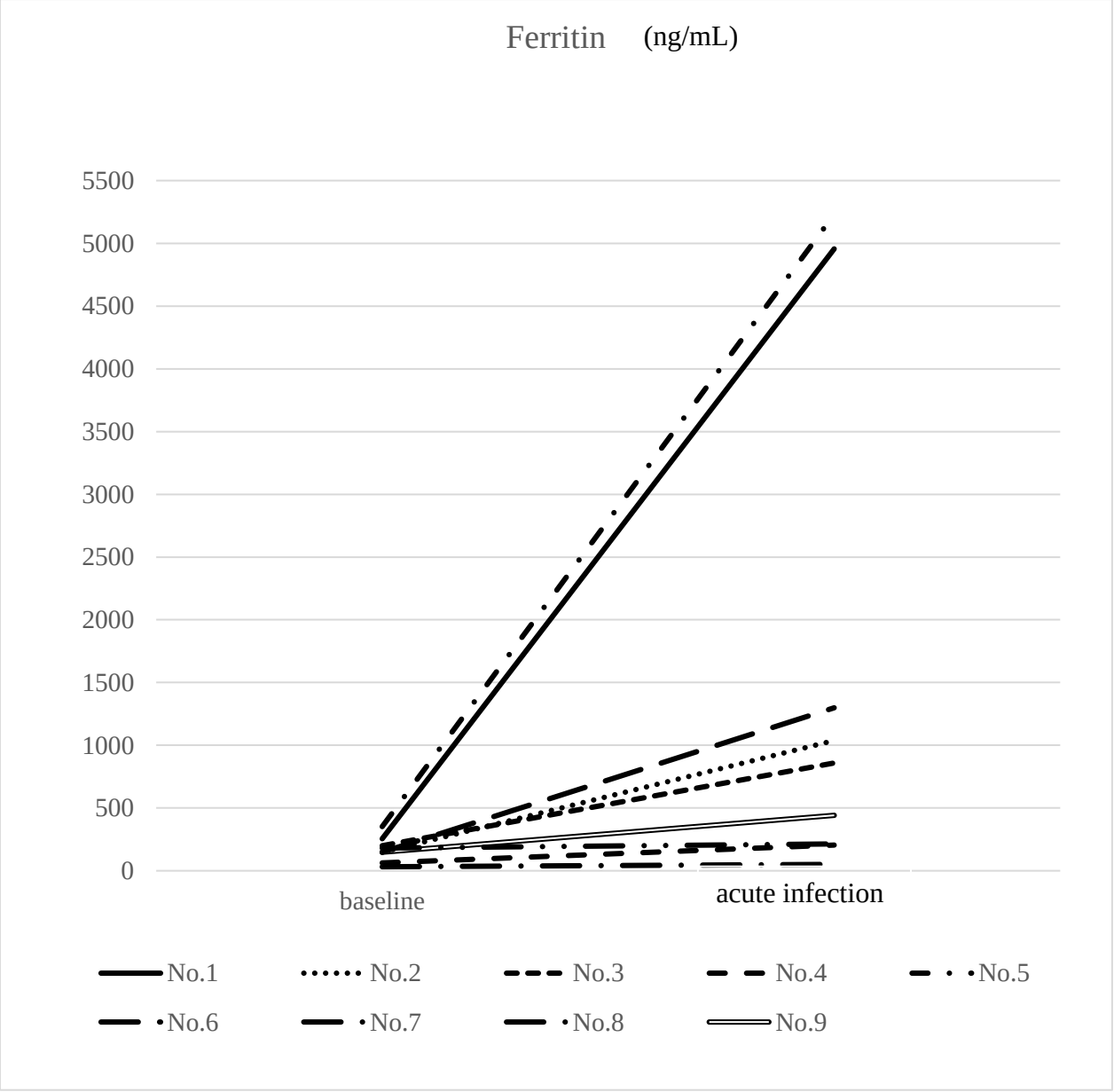

Figure 6. Ferritin change in active SLE patients with bacterial infection

During the acute infection phase, the mean serum ferritin levels in active SLE patients were above 600ng/mL.

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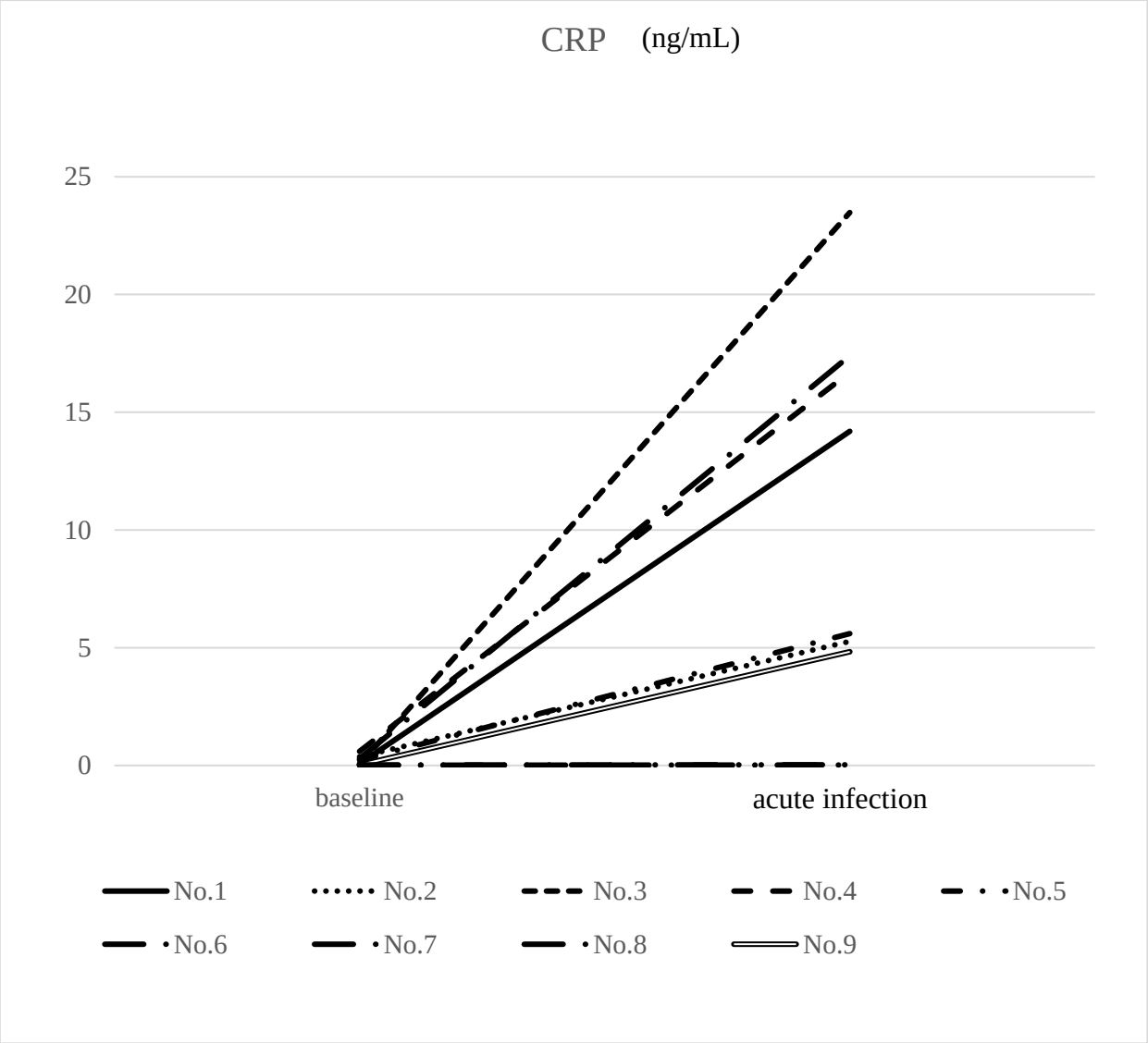

Figure 7. CRP change in active SLE patients with bacterial infection

During the acute infection phase, the mean serum CRP levels of active SLE patients were above 5mg/dL.

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The Receiver Operating Characteristic (ROC) Curve

For active SLE patients, we calculated the ROC curve to find the best cut-off level of serum ferritin in infection and found that the best level for the prediction of infection is ≥600ng/mL (400-799ng/mL) (sensitivity:73% specificity:95.23) (**Table 4**) (*see* **Figure 8**).

Table 4. ROC Curve Calculations

	Observed		Cumulative		False Positive Rate	True Positive Rate	Area under the ROC Curve
Data value	Pass (Noninfected)	Fail (Infected)	Pass (Noninfected)	Fail (Infected)	FPR	TPR	AUC
			0	0	1	1	0.857142857
0-399	18	3	18	3	0.142857143	0.8	0.076190476
400-799	2	1	20	4	0.047619048	0.733333333	0.034920635
800-1199	1	5	21	9	0	0.4	0
1200-1599	0	1	21	10	0	0.333333333	0
>1600	0	5	21	15	0	0	0
Sum	21	15					
		Total 36					0.968253968

A total of eighteen active SLE patients (infected n=15, non-infected n=3) were enrolled. Thirty-six ferritin data points were recorded (baseline n=18, acute infection n=18).

Null hypothesis was the non-infected. We found that the best prediction model for serum ferritin level against the null hypothesis was within 400-799 ng/mL (mean 600ng/mL).

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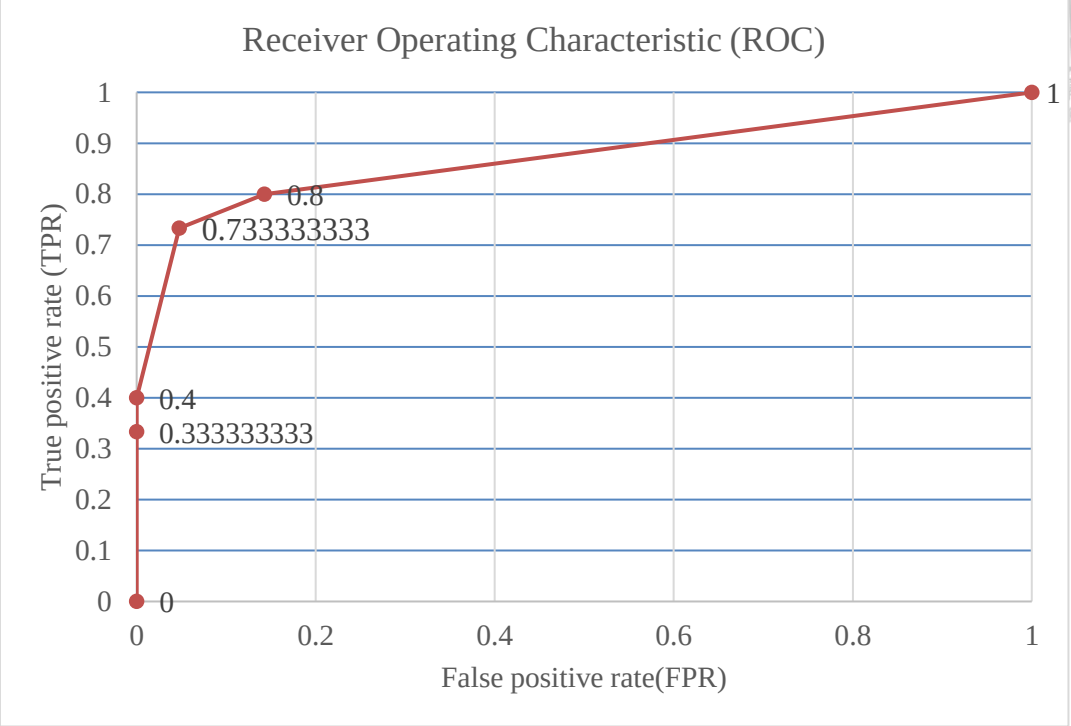

Figure 8. ROC curve analysis for serum ferritin in active SLE patient with infection

The true positive rate (sensitivity) is on the y axis and false positive rate (1−specificity) is on the x axis. The ideal threshold was within 400-799 ng/mL (mean 600ng/mL).

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Glycosylated ferritin (GF) analysis

The results of serum glycosylated ferritin levels are shown in **Table 5, 6** and **Figure 9**.

Table 5. Glycosylated Ferritin (GF) ELISA Results

Sample ID	Location	(OD) Data	(OD) Mean	S.D.	C.V.	Dilution	(ng/mL) Conc.
T1	H1	1.540	1.540	*****	*****	1	39.262#
T2	A2	0.961	0.961	*****	*****	1	53.956#
T3	B2	1.112	1.112	*****	*****	1	48.893#
T4	C2	0.883	0.883	*****	*****	1	57.107#
T5	D2	0.969	0.969	*****	*****	1	53.658#
T6	E2	0.739	0.739	*****	*****	1	64.379#
T7	F2	0.572	0.572	*****	*****	1	76.535
T8	G2	1.516	1.516	*****	*****	1	39.670#
T9	H2	1.360	1.360	*****	*****	1	42.694#
T10	A3	0.869	0.869	*****	*****	1	57.721#
T11	B3	1.109	1.109	*****	*****	1	48.970#
T12	C3	0.993	0.993	*****	*****	1	52.778#
T13	D3	0.720	0.720	*****	*****	1	65.544#
T14	E3	1.360	1.360	*****	*****	1	42.690#
T15	F3	1.772	1.772	*****	*****	2	71.438#
T16	G3	2.273	2.273	*****	*****	4	120.771#
T17	H3	1.448	1.448	*****	*****	2	81.826#
T18	A4	2.206	2.206	*****	*****	4	123.233#

Sample ID	Location	(OD) Data	(OD) Mean	S.D.	C.V.	Dilution	(ng/mL) Conc.
S1	A1	0.040	0.040	*****	*****	1	2400.000
S2	B1	0.038	0.038	*****	*****	1	1200.000
S3	C1	0.065	0.065	*****	*****	1	600.000
S4	D1	0.143	0.143	*****	*****	1	300.000
S5	E1	0.211	0.211	*****	*****	1	150.000
S6	F1	0.589	0.589	*****	*****	1	75.000

All the active SLE patients with acute infection displayed low glycosylated ferritin/ferritin ratio, which indicated that the hyperferritinemia may have resulted from SLE reaction rather than cell damage. However, systemic errors such as sample decay and technology error may have contributed to the same results.

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Table 6. Glycosylated Ferritin (GF)/Ferritin ratio

Patient No	Event	Ferritin(ng/mL)	GF	GF/Ferritin ratio
No.01	SLE LN, Cryptococcus	7255	39.26	0.54%
No.02	SLE w/ SSc, Cryptococcus	165	53.96	32.70%
No.05	SLE LN, E.Coli UTI	214	53.66	25.07%
No.11	SLE LN, E.Coli UTI	442	48.97	11.08%
			Mean	**17.35%**
No.09	SLE LN, Flare	83	42.69	51.43%
N0.10	SLE LN, Flare	583	57.72	9.90%
No.14	SLE LN, Flare	74	42.69	57.69%
			Mean	**39.67%**

SLE: Systemic Lupus Erythematosus, LN: Lupus nephritis, SSc: Systemic sclerosis

As shown in **Table 6**, the GF/Ferritin ratios were lower in the SLE with infection group (mean **17.35%**, 0.54 - 32.70) than the disease flare group (**39.67%**, 9.9 - 57), which indicated that the result of hyperferritinemia in active SLE patients with infection may be due to overexpression of inflammasome pathway rather than cell damage/lysis.

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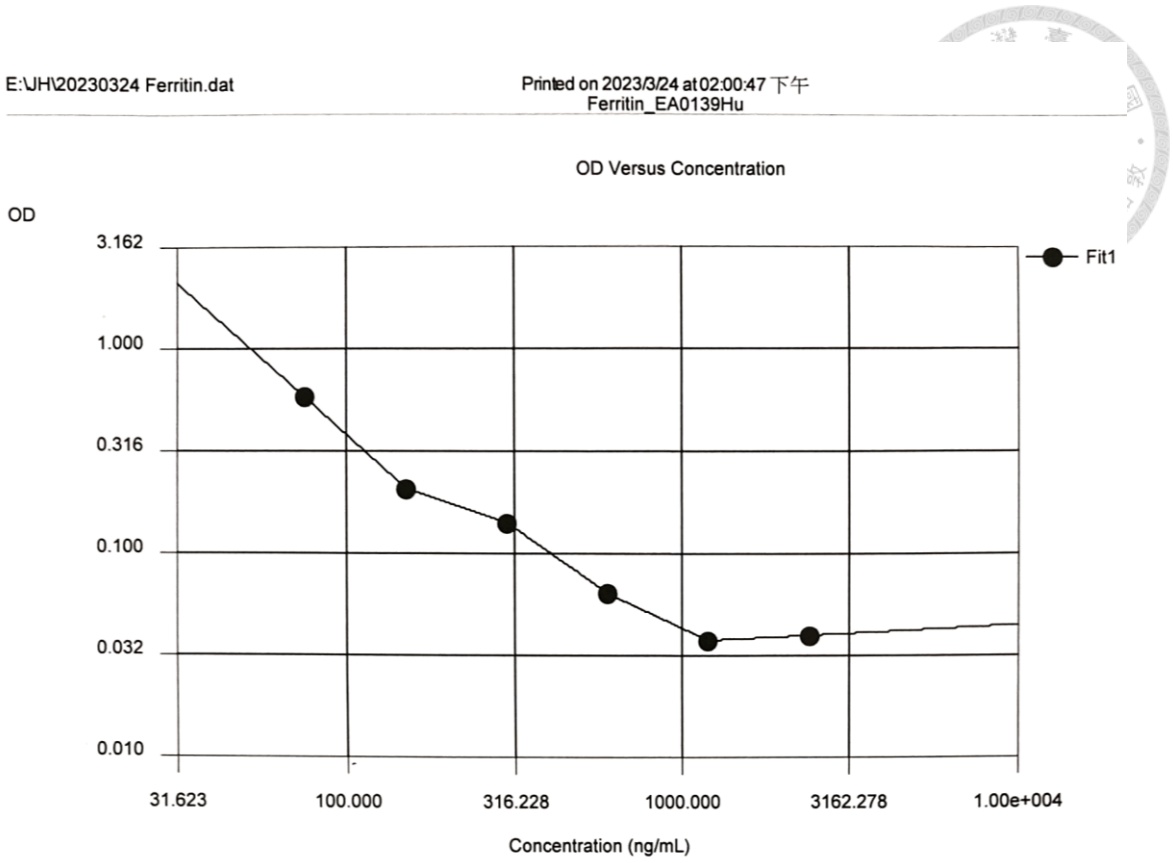

Figure 9. Glycosylated Ferritin ELISA Standard Curve

The standard curve was very consistent.

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Discussion

The results of this study showed that when CRP is ineffective in detecting early infection in active SLE patients, elevating serum ferritin can serve as a reliable marker.

As shown in **Table 7** below, compared to previous studies [25](Cengić, M., et al. 2002) for CRP levels in active SLE patients with atypical and bacterial infection, the ferritin levels for most patients with atypical infection and bacterial infection were above 600 ng/mL, whereas only the CRP level for bacterial infection was above 6 mg/dL.

Additionally, the sensitivity and specificity for SLE patients with infection and ferritin levels ≥ 600ng/mL were 73% and 95.23%, respectively, and for CRP ≥6.0mg/dL, 82.4% and 66.55%, respectively. CRP displayed limited elevation in active SLE patients with atypical infection (CRP level increase was ineffective), which may have been due to the overactivity of the inflammasome pathway. The low GF/F ratio (mean **17.35%**) of active SLE patients with infection discussed in **Table 6** confirmed this hypothesis.

Table 7. Infection markers for SLE patients

	Ferritin (ng/mL)	CRP (mg/dL)
Atypical infection	**≥ 600**	ineffective
Bacterial infection	≥ 600	≥6.0
Sensitivity	73%	82.4%
Specificity	95.23%	66.55%

In Group 1 active SLE with atypical infection, the baseline mean ferritin (423ng/mL) appeared higher than previous studies (mean ferritin: 245.3ng/mL)[30]

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(Tripathy, R., A.K. Panda, and B.K. Das., et al. 2015). This may be attributed to the fact that one patient had a high baseline ferritin (1038ng/mL). But all of the patients exhibited significant elevation of ferritin (*see* **Figure 4**).

In Group 4 other autoimmune diseases with bacterial infection(n=3), the mean acute phase ferritin was very high (17407ng/mL) due to one patient with severe septic shock (ferritin: 30865 ng/mL). The other two patients' serum ferritin levels in acute phase are 365 and 995 ng/mL, respectively.

The reason why the results from the group with the other autoimmune diseases with bacterial infection were not statistically significant may have been due to the limited case number in this study (n=3).

Conclusion

Taking together all the results of the current literature and our study, we found that serum ferritin level (≥ 600ng/dL) can serve as an infection marker to monitor active SLE with both atypical and bacterial infection, while the CRP level may only serve as a tool for active SLE with bacterial infection. The result of hyperferritinemia in active SLE patient with infection may due to SLE reaction rather than cell damage/lysis due to low GF/F ratio. Therefore, clinically when it is difficult to differentiate between SLE patients with infections or disease flare, testing both ferritin and CRP can assist practitioners make a more precise diagnosis.

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Limitation

The main limitations of this study include the limited number of cases, long-term storage, blood test delay, and occult multiple infections.

Due to the limited number of patients that fit the study's profile, the number of cases only amounted to 25 infectious events in 21 patients. Furthermore, for Group 4 other autoimmune diseases with bacterial infection, we were only able to collect 3 cases, which limited the significance of the results.

Systemic errors like decay of serum glycosylated ferritin due to long term storage (over two years) and possible temperature drop during the sample transport process may have also attributed to the results of the serum glycosylated ferritin (39-123ng/mL), which were consistency low. Thus, we will need fresher samples in future studies to confirm our results.

Furthermore, because of the clinical difficulty to identify infection early in SLE patients, the delay of blood tests ordered and performed could also have resulted in serum glycosylated ferritin decay.

Finally, patients with active SLE tend to encounter multiple infections. However, the detection for virus pathogen is not routine in clinical practice for septic workups. Therefore, multiple infections that are not recognized could also interfere with the analysis of our results.

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3. 附錄(Appendix)

3.1 SLICC 2012 Criteria

Petri, M., et al., Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum, 2012. 64(8): p. 2677-86[35]

3.2 SLEDAI 2k score

Zahi Touma, et al., Systemic Lupus Erythematosus Disease Activity Index 2000 Responder Index 50: sensitivity to response at 6 and 12 months Rheumatology, Volume 51, Issue 10, October 2012, Pages 1814–1819

3.3 SLEDAI 2k score and SLE activity

Dafna D Gladman et al., Systemic lupus erythematosus disease activity index 2000The Journal of Rheumatology, 2002. 29(2): p. 288-291.

3.4 Competitive ELISA

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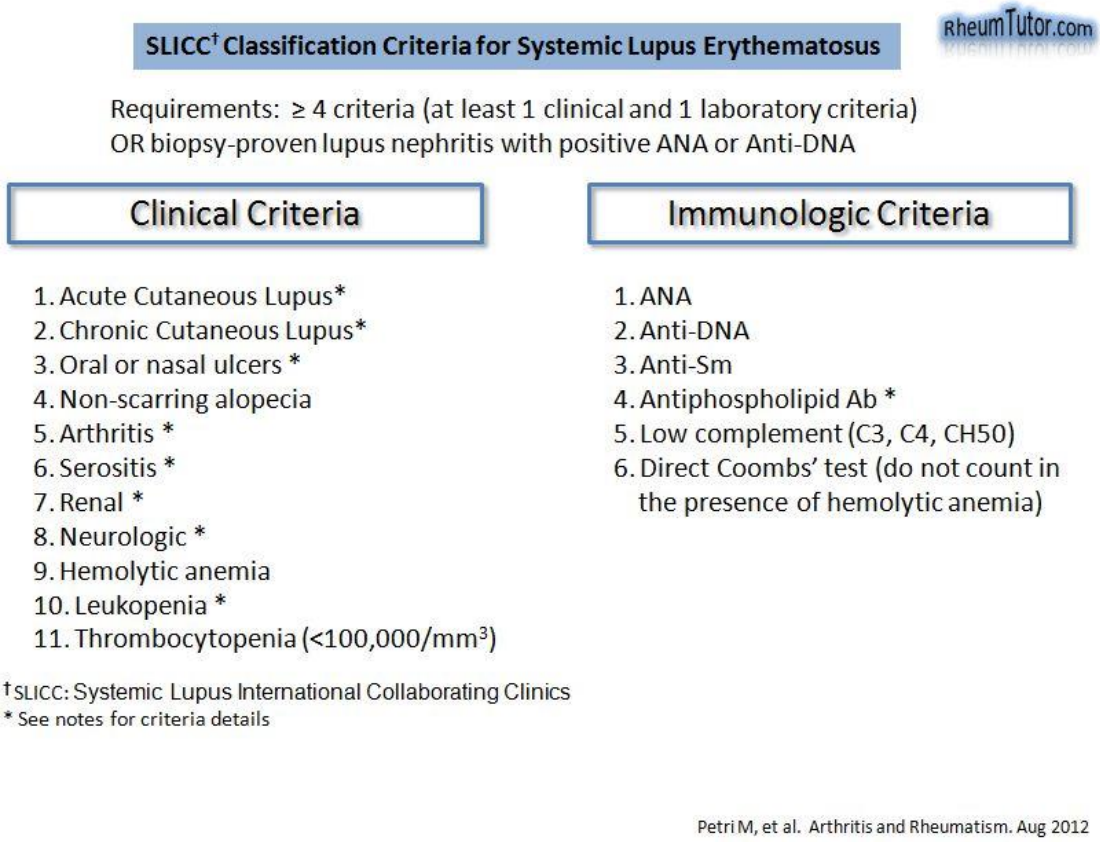
附錄 1. SLICC 2012 Criteria



SLICC 2012 criteria for SLE diagnosis

SLICC 2012 criteria comprises clinical and immunologic part. Most common clinical signs and symptoms of SLE were involved. It has good sensitivity(97%) and acceptable specificity(84%).

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附錄 2. SLEDAI 2k Score

SLEDAI-2K score	Descriptor	Definition
8	**Seizure**	Recent onset, exclude metabolic, infectious or drug causes.
8	**Psychosis**	Altered ability to function in normal activity due to severe disturbance in the perception of reality.
8	**Organic brain syndrome**	Altered mental function with impaired orientation, memory or other intellectual function.
8	**Visual disturbance**	Retinal changes.
8	**Cranial nerve disorder**	New onset of sensory or motor neuropathy involving cranial nerves.
8	**Lupus headache**	Severe, persistent headache which may be migrainous, but must be nonresponsive to narcotic analgesia.
8	**Cerebrovascular accident**	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8	**Vasculitis**	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter haemorrhages, or biopsy or angiogram proof of vasculitis.
4	**Arthritis**	≥2 joints with pain and signs of inflammation (i.e. tenderness, swelling or effusion).
4	**Myositis**	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or biopsy showing myositis.
4	**Urinary casts**	Heme granular or red blood cell casts.
4	**Haematuria**	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4	**Proteinuria**	>0.5 gram/24 hours.
4	**Pyuria**	>5 white blood cells/high power field. Exclude infection.
2	**Rash**	Inflammatory type rash.
2	**Alopecia**	Abnormal, patchy or diffuse loss of hair.
2	**Mucosal ulcers**	Oral or nasal ulcerations.
2	**Pleurisy**	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	**Pericarditis**	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.
2	**Low complement**	Decrease in CH50, C3 or C4.
2	**Increased DNA binding**	Increased DNA binding by Farr assay.
1	**Fever**	>38°C. Exclude infectious cause.
1	**Thrombocytopenia**	<100 000 platelets / $x10^9$/L, exclude drug causes.
1	**Leukopenia**	<3000 white blood cells / $x10^9$/L, exclude drug causes.

C3 = Complement protein 3, C4 = Complement protein 4, CH50 = 50% haemolytic complement activity, DNA = deoxyribonuclease, SLEDAI-2K = SLE disease activity index 2000

Summarized from *Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol. 2002;29:288-91* (99).

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附錄 3. SLEDAI 2k Score and SLE Activity

Activity Level	No. (%)	SLEDAI*	SLEDAI-2K*	p
0–No activity	465 (43.9)	1.95 (2.29) [2]	2.23 (2.34) [2]	0.066
1–Mild activity	231 (21.8)	3.80 (2.85) [4]	4.42 (2.93) [4]	0.023
2–Activity, but improvement	87 (8.2)	6.74 (3.93) [6]	7.45 (3.93) [8]	0.233
3–Persistent activity	165 (15.6)	7.78 (4.05) [8]	8.80 (4.05) [8]	0.023
4–Flare	112 (10.6)	9.37 (6.13) [9]	9.76 (6.15) [9]	0.632

*Mean score (SD) [median].

Gladman, D.D., D. Ibañez, and M.B. Urowitz, Systemic lupus erythematosus disease activity index 2000. The Journal of Rheumatology, 2002. 29(2): p. 288-291.

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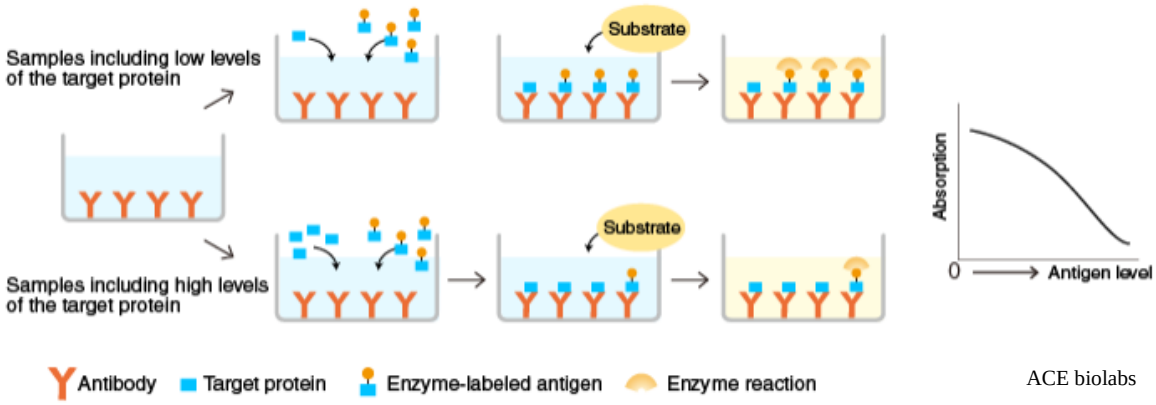
附錄 4. Competitive ELISA



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The more antigens in the sample, the more Ag-Ab complexes are formed, causing less unbound antibodies available to bind to the antigen in the well, hence “competition”

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