Differential Parameters between Activity Flare and Acute Infection in Pediatric Patients with Systemic Lupus Erythematosus

Kai-Ling Luo

Cathay General Hospital

Yao-Hsu Yang

National Taiwan University Hospital

Yu-Tsan Lin

National Taiwan University Hospital

Ya-Chiao Hu

National Taiwan University Hospital

Hsin-Hui Yu

National Taiwan University Hospital

Li-Chieh Wang

National Taiwan University Hospital

Bor-Luen Chiang

National Taiwan University Hospital

JYH-HONG LEE (✉ leonid@ntu.edu.tw)

National Taiwan University Hospital <https://orcid.org/0000-0003-4285-4912>

Research article

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Abstract

Background:

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease. SLE patients are vulnerable to infections. Infections might mimic and even trigger SLE flares. To distinguish acute infection from activity flare always remains a clinical challenge. We aim to explore the approach to differentiate active infection from disease activity in pediatric SLE patients.

Methods:

Fifty pediatric SLE patients presenting with 185 clinical episodes were collected. The associations between relevant data encompassing both clinical and laboratory parameters and the outcome groups were analyzed using generalized estimating equations (GEEs) facilitating the analysis of data collected in longitudinal and repeated-measures designs. We also used a multinomial logistic regression model for simultaneous prediction of different outcome groups. Finally, we ran the GLIMMIX procedure to track trends of changes in parameters over time.

Results:

These 185 episodes were divided into 4 outcome groups: infected-active (n=102), infected-inactive (n=11), noninfected-active (n=59), and noninfected-inactive (n=13) episodes. Multivariate GEE analysis showed that SDI, SLEDAI-2K, neutrophil-to-lymphocyte ratio (NLR), hemoglobin, platelet, RDW-to-platelet ratio (RPR), and C3 are predictive of flare (combined calculated AUC of 0.8964 and with sensitivity of 82.2% and specificity of 90.9%). Multivariate GEE analysis showed that SDI, fever temperature, CRP, procalcitonin, lymphocyte percentage, NLR, hemoglobin, and renal score in SLEDAI-2k are predictive of infection (combined calculated AUC of 0.7886 and with sensitivity of 63.5% and specificity of 89.2%). We developed regression equations for simultaneous prediction of 4 different outcome groups. We could differentiate noninfected-active from infected-active episodes by different change patterns of ESR, NLR, lymphocyte, C3, and C4 over time.

Conclusions:

The proposed approach could differentiate flares from infections in pediatric SLE patients. Combination of parameters from four different domains simultaneously, including inflammation (CRP, ESR, PCT), hematology (Lymphocyte percentage, NLR, PLR), complement (C3, C4), and clinical status (SLEDAI, SDI), is effective to make appropriate judgement and treatment decisions.

Background

Systemic lupus erythematosus (SLE) is an autoimmune disease caused by autoreactive B cells in combination with T cell dysregulation and cytokine abnormalities [1]. The presentation, disease course, and outcomes of SLE are unpredictable. Approximately 60–70% of patients exhibit relapsing-remitting

and active disease patterns [2]. Pediatric lupus patients typically have a more severe disease [3]. Despite the more frequent use of corticosteroids and immunosuppressive agents, a significantly higher percentage of children with SLE continue to have a state of high disease activity in comparison to adults [3]. Several indices have been designed to assess disease activity. The most commonly used disease activity score is the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [4]. The Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI) is a reliable instrument for the assessment of degree of disease-related damage in children with SLE [5]. SLE can also be complicated by chronic multi-organ damage and infections, which are difficult to distinguish from lupus flares due to their similar clinical features. Most such studies to date were performed in adult populations, and data regarding pediatric SLE are lacking.

Infections are a major cause of morbidity and mortality in SLE patients. SLE patients are highly susceptible to infections due to the combined effects of immunosuppressive therapy and abnormalities of the immune system. In Taiwan, infections are among the leading causes of death in pediatric SLE [6]. Fever is a common symptom in pediatric SLE, and it is difficult to distinguish between an SLE flare and febrile infection [7]. Some infections may produce systemic manifestations mimicking SLE, either superimposed upon or triggering a flare [8]. The interaction between infection and SLE is complicated, as viral, bacterial, parasitic, and fungal pathogens can trigger SLE disease activity through molecular mimicry [9]. The establishment of a causative link between infection and autoimmunity has been studied in detail, with the role of infectious agents in the induction as well as the progression or exacerbation of SLE confirmed [10]. In some patients, both activity flare and acute infection can coexist, making the diagnosis and therapeutic approach challenging. A delay in antimicrobial therapy of > 24 h reportedly increased the mortality of hospitalized SLE patients 12-fold; therefore, early identification and treatment of infections are essential [11]. Clinicians have to make treatment decisions based on clinical judgment and laboratory parameters to distinguish between active disease and infection.

There have been a number of studies on the predictive biological markers of SLE flares, including anti-double-stranded DNA antibodies (anti-dsDNA Ab), the complement system, anti-extractable nuclear antigen antibodies (anti-ENA Ab), cytokines, and chemokines [12, 13]. For the prediction of infection in SLE patients, conventional biomarkers (C-reactive protein [CRP], erythrocyte sedimentation rate (ESR), procalcitonin [PCT])[14–18] and new markers have been developed [19]. Several recent studies have focused on markers for differentiating between disease flare and infection in febrile SLE patients [20–22]. However, most physicians agreed that no single biomarker had sufficient predictive value for both events [8, 19]. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were significantly higher in SLE patients compared with healthy controls and were positively correlated with SLEDAI score [23]. However, the NLR could be a good additive marker for diagnosing infection in patients with SLE [24]. New scores, which include combinations of different biomarkers, may represent better solutions for differentiation [19].

It is possible that the use of only one biomarker would not be enough to confirm or disregard infection from activity. We aimed to identify useful parameters for the differential diagnosis of disease flares and

infections in pediatric-onset SLE patients, and to develop predictive calculators that could assist in decision-making in daily clinical practice.

Materials And Methods

Patients

This was a retrospective study conducted by reviewing the medical records of 50 pediatric-onset (≤ 18 years-old) SLE [25] patients presenting with 185 clinical events (episodes) for any clinical condition from August 1, 2015, to September 1, 2019, at the Department of Pediatrics, National Taiwan University Hospital (NTUH). Patients who did not meet the 1997 ACR criteria for SLE diagnosis, and patients with overlapping autoimmune diseases or other chronic inflammatory diseases/infections or malignancies were excluded. This study was approved by the Institutional Review Board and Ethics Committee of NTUH and was conducted in compliance with the protocol for good clinical practices and the principles of the Declaration of Helsinki.

Based on previous reports [23, 26–29], data encompassing both clinical and laboratory parameters were collected at each episode according to a standardized clinical protocol (detailed in **supplement** Table 1).

Table 1
Characteristics, clinical manifestations, infections
patterns, and medications of 50 pediatric SLE
patients.

PATIENTS	n = 50
Age (years) at enrollment	13.9 ± 4.4
Male:Female, n (%)	7:43 (14:86)
Organ involvement during flare episodes, n (%)	
Neuropsychiatric	11 (22)
Vasculitis	9 (18)
Arthritis	24 (48)
Myositis	2 (4)
Renal	36 (72)
Mucocutaneous	36 (72)
Serositis	15 (30)
Hematologic	21 (42)
Specific infections, n (%)	
Respiratory	28 (56)
Skin and soft tissue	4 (8)
Infective endocarditis	1 (2)
Brain abscess	1 (2)
Intra-abdominal infection	5 (10)
Urinary tract infection	10 (20)
Bacteremia	5 (10)
Herpes zoster	3 (6)
Identified pathogens, n (%)	
Bacterial	15 (30)
Viral	6 (12)
Fungal	10 (20)
Bacterial + Fungal	6 (12)
Previous medications, n (%)	

PATIENTS	n = 50
Hydroxychloroquine	35 (70)
Oral glucocorticoid	38 (76)
Immunosuppressant	34 (68)
Cyclosporine	12 (24)
Azathioprine	8 (16)
Mycophenolic	18 (36)
Medications during admission, n (%)	
Steroid pulse therapy	17 (34)
Steroid mini-pulse therapy	17 (34)
Cyclophosphamide pulse therapy	9 (18)
Rituximab	4 (8)

Outcome definitions

Disease flare was defined as: (1) the addition of or increase in the dose of corticosteroids within 2 weeks; and/or (2) the addition of an immunosuppressive medication (cyclophosphamide, cyclosporine, azathioprine, or mycophenolate mofetil) within 2 weeks; and/or (3) an increase in SLEDAI score within 2 weeks, with an increase > 3 points considered a mild or moderate flare, and an increase > 12 points considered a severe flare. If an immunosuppressive medication was added as a steroid-sparing agent, and not because of active disease, the visit was not considered to be due to a flare [30, 31].

Acute infections was defined based on: (1) microbiological culture/isolation evidence that explained clinical symptoms; (2) clinical symptoms and/or inflammatory syndrome and/or laboratory/serological results that rapidly regressed after starting antimicrobial (antibiotic, antiviral, or antifungal) therapy; (3) confirmation by radiological and/or imaging study; and/or (4) confirmation via an infectious specialist consultation [20, 21, 32, 33].

Statistical analysis

The clinical and laboratory characteristics of the four groups were analyzed. Quantitative variables are presented as means $\pm$ standard deviations and ranges. Qualitative variables are shown as numbers (*n*) and percentages. The Mann–Whitney test was used instead of the *t*-test for the analysis of non-parametric data. The associations between parameters and the four outcome groups were analyzed using univariate and multivariate generalized estimating equations (GEEs) with an autoregressive (AR) model as the GEE approach facilitates the analysis of data collected in longitudinal, nested, or repeated-measures designs and produces more efficient and unbiased regression estimates for analyzing measures with non-normal response variables [34]. For each input parameter, univariate logistic

regressions were then fit to specific outcome models. Parameters that were considered significant in univariate analyses at $P < 0.05$ were defined as “informative”. Informative parameters were tested together as covariates in a stepwise multivariate analysis. The remaining parameters were designated as “significant effectors” of activity flare or acute infection and were used to derive a prediction score equation.

SLE is characterized by a relapsing-remitting course between at least two discrete clinical/laboratory episodes, separated by periods of clinical quiescence [35]. Therefore, we assumed that the discrete episodes were all conceptually independent. We thus used a multinomial logistic regression model for nominal outcomes [36], by setting group D (noninfected-inactive group) as the reference group, and developed three regression equations for simultaneous prediction. The relationship between the other outcome (for group A, B, C) and any particular explanatory variable (clinical and laboratory parameters) is now captured using particular parameters which define the log odds of response jumping from the reference outcome (group D) to the otherwise outcome (group A, B, C) [36].

There were 24 hospitalized patients present 29 admissions with serial measurement of parameters during admission. The evaluation at the first time point provided baseline data, and the data collected at each following time point were assigned into one of the following 10 time periods: baseline/day 0 (assigned as time point 0), < 3 days (time point 1), 3–5 days (time point 2), 6–10 days (time point 3), 11–15 days (time point 4), 16–20 days (time point 5), 21–25 days (time point 6), 26–30 days (time point 7), 31–35 days (time point 8), and 36–40 days (time point 9). To identify potential markers, we analyzed group-specific parameter trends; i.e., baseline data and data from subsequent assessments in groups A vs C using a multivariate GEE with the AR model and the GLIMMIX procedure. To track trends in changes in laboratory and clinical parameters in groups A and C over time, we ran the GLIMMIX procedure using 13 variables, employing verification steps such as the type III tests of fixed effects to provide a solution for fixed effects. The analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Results

Patient and clinical characteristics

Fifty patients account for a total 185 times of clinical episodes were included in the study (Table 1). Among these 50 patients, 7 (14%) were male and 43 (86%) were female, and the mean age at enrollment was 13.9 ± 4.4 years old. These 185 episodes were divided into 4 groups: infected-active episodes as group A ($n = 102$; 55%), infected-inactive episodes as group B ($n = 11$; 6%), noninfected-active episodes as group C ($n = 59$; 32%), and noninfected-inactive episodes as group D ($n = 13$; 7%) (Table 2). In the similar studies of adult SLE patients comparing parameters from the four groups, the trend of our CRP results is the same as three reported literatures [18, 28, 32]. While the trend of ESR and PCT are also in consistent with those of Schäfer *et al.* and Wang *et al.*, respectively (**supplement Fig. 1**). Like the result from Broca-Garcia *et al.*, infected-active group (group A) had the highest values of PLR among all four groups [28].

Our data is in consistent with Aringer *et al.* stating that, without infection, CRP levels are higher in active SLE than in inactive SLE, the opposite is true in infections in SLE patients [18, 37].

Table 2
Comparison 185 clinical episodes related to activity flare and/or acute infection.

Group (n = 185)	infected- active (n = 102)	infected- inactive (n = 11)	noninfected- active (n = 59)	noninfected- inactive (n = 13)
LABORATORY PARAMETERS (mean ± SD)				
WBC (/uL)	8430 ± 5807	6702 ± 3717	8441 ± 5793	10715 ± 5313
Hb (g/dL)	9.2 ± 1.9	11.4 ± 2.3	10.7 ± 2.0	11.9 ± 1.9
PLT (x10 ³ /uL)	176.8 ± 114.6	224.5 ± 86.0	229.5 ± 135.8	310.6 ± 100.6
Segment (%)	77.7 ± 17.8	77.6 ± 9.5	72.8 ± 14.3	81.2 ± 7.5
Lymphocyte (%)	12.9 ± 11.6	16.3 ± 8.4	19.9 ± 12.7	12.3 ± 5.2
CRP (mg/dL)	2.25 ± 4.08	2.32 ± 2.27	1.02 ± 2.33	0.31 ± 0.42
ESR (mm/hr)	41.0 ± 38.4	38.8 ± 18.6	33.5 ± 32.3	29.7 ± 19.5
ESR/CRP	168.7 ± 433.6	94.2 ± 178.5	254.5 ± 387.0	271.5 ± 366.0
Procalcitonin (ng/mL)	1.998 ± 6.232	0.54 ± 1.18	0.27 ± 0.44	0.06 ± 0.04
ANA titer	1,303 ± 940	352 ± 476	1,187 ± 966	467 ± 578
C3 (mg/dL)	62.71 ± 31.45	78.18 ± 30.50	58.96 ± 37.94	103.41 ± 24.75
C4 (mg/dL)	13.67 ± 10.09	18.90 ± 10.85	11.23 ± 9.37	13.18 ± 6.17
anti-dsDNA (IU/mL)	545.18 ± 492.99	601.38 ± 330.58	770.89 ± 418.78	291.57 ± 310.66
NLR	22.19 ± 37.09	8.07 ± 7.66	9.47 ± 19.40	8.73 ± 5.40
PLR	421.0 ± 396.5	302.0 ± 128.8	253.1 ± 177.2	305.0 ± 98.1
RPR	0.15 ± 0.14	0.07 ± 0.02	0.11 ± 0.13	0.06 ± 0.03
CLINICAL SCORING INDICES				
SLEDAI-2K (mean ± SD)	19.68 ± 8.30	9.64 ± 5.53	15.53 ± 8.29	10.15 ± 5.29
mild activity (<= 6), (n, %)	4 (7%)	4 (36%)	9 (15%)	5 (38%)
moderate activity (7–12), (n, %)	17 (19%)	3 (28%)	17 (29%)	4 (31%)
severe activity (> 12), (n, %)	76 (74%)	4 (36%)	33 (56%)	4 (31%)
Renal column (mean)	9.81	3.64	5.69	7.69
CNS column (mean)	2.78	1.09	0.68	0
Vasculitis column (mean)	0.86	0	1.49	0.31

Group (n = 185)	infected-active (n = 102)	infected-inactive (n = 11)	noninfected-active (n = 59)	noninfected-inactive (n = 13)
Arthritis column (mean)	0.71	0.36	1.42	0.31
Myositis column (mean)	0.24	0	0	0
Cutaneous column (mean)	0.85	1.27	1.83	0.62
Serositis column (mean)	1.02	0	0.51	0
Complement column (mean)	1.62	0.73	1.66	0.77
SLICC/ACR Damage Index (SDI) (mean, range)	3.01 (0–7)	0.64 (0–2)	0.69 (0–5)	0.92 (0–3)

Parameters predictive of activity flare

Among all the parameters analyzed, we found SDI score, SLEDAI 2K score, NLR, RDW-to-platelet ratio (RPR), ANA levels, anti-dsDNA levels, antiphospholipid Ab levels, and urine cast were positive predictors of activity flare. But that Hb levels, platelet levels, C3 levels, C4 levels, and urine nitrate were negatively associated with the occurrence of disease activity (**supplementary Table 1a**). Multivariate GEE analysis showed that SDI score, SLEDAI 2K score, NLR, Hb levels, platelet levels, RPR, and C3 levels were independent parameters for predicting SLE activity flare (Table 3a). Here we confirmed that NLR, PLR, and RPR is a useful marker for the assessment of disease activity in pediatric SLE patients [23, 29]. Combination of these seven parameters resulted in a model with calculated AUC of 0.8964 and with sensitivity of 82.2% and specificity of 90.9% (Fig. 1a).

Table 3

a: Multivariate GEE for outcome of activity flare. Seven significant effectors are shown.

Parameter	Estimate	Standard Error	95% Confidence Limits		P-value
SDI	-0.0148	0.0088	-0.0320	0.0025	0.0936
SLEDAI 2K	0.0108	0.0029	0.0050	0.0165	0.0002
NLR	0.0013	0.0004	0.0005	0.0021	0.0010
Hb	-0.0305	0.0126	-0.0552	-0.0057	0.0159
PLT*	-0.0147	0.0265	-0.0667	0.0373	0.5797
RPR	0.1614	0.2026	-0.2357	0.5585	0.4257
C3	-0.0025	0.0013	-0.0050	-0.0001	0.0449
*: original values divided by 100.					
NLR: neutrophil-to-lymphocyte ratio; RPR: RDW-to-platelet ratio.					

Table 3

b: Multivariate GEE for outcome of acute infection. Eight significant effectors are shown.

Parameter	Estimate	Standard Error	95% Confidence Limits		P-value
SDI	0.0782	0.0169	0.0451	0.1114	< .0001
Fever temperature	0.0997	0.0947	-0.0859	0.2853	0.2926
CRP	0.0341	0.0075	0.0195	0.0487	< .0001
PCT	0.0005	0.002	-0.0035	0.0045	0.8069
Lymphocyte percentage	-0.0006	0.0034	-0.0073	0.0061	0.8520
NLR	0.0005	0.0007	-0.0009	0.0019	0.4840
Hb	-0.0125	0.0243	-0.0601	0.0352	0.6086
SLEDAI 2K renal score	0.0086	0.0079	-0.0069	0.0242	0.2769
NLR: neutrophil-to-lymphocyte ratio; PCT: procalcitonin.					

We then propose an Activity Predict Score formula:

Activity Predict Score = $1.1707 - 0.0146 \cdot \text{SDI score} + 0.0108 \cdot \text{SLEDAI 2K score} + 0.0013 \cdot \text{NLR} - 0.0305 \cdot \text{Hb} - 0.0147 \cdot \text{PLT (original value divided by 100)} + 0.1614 \cdot \text{RPR} - 0.0025 \cdot \text{C3}$.

We obtained the largest Youden Index when the cut-off point of Activity Predict Score is cut at 0.76652; that is, when Activity Predict Score is greater than 0.76652, it will be classified to be an activity flare; if it is

less than 0.76652, it will be classified as without activity flare.

Parameters predictive of acute infection

Using GEE, we found acute infection were associated with SDI score, fever temperature, CRP levels, PCT levels, NLR, PLR, and renal score of SLEDAI 2K while lymphocyte percentage, Hb levels, and urine nitrate were negative predictors of infectious events (**supplementary Table 1b**). Multivariate GEE analysis showed that SDI score, fever temperature, CRP level, PCT levels, lymphocyte percentage, NLR, Hb levels, and SLEDAI 2K renal score are independent parameters for predicting acute infection in SLE patients (Table 3b). Renal disease, despite being associated with infections in the univariate analysis, has not retained the statistical significance in the multivariate analysis in some series [38, 39]. However, our result confirmed that renal involvement is significantly associated with active infection in the multivariate GEE analysis, in consistent to previous study [40]. Of note, our data is in consistent with Pimentel *et al.* that any increase in the SDI was associated with the occurrence of serious infections [39]. We also showed that compared to PCT, CRP is a more sensitive and specific marker for diagnosing bacterial infections in SLE [41]. However, there had also been some reports that PCT is more specific and has better diagnostic accuracy than CRP for infection in SLE [15, 33, 42]. Combination of these eight parameters resulted in a model with calculated AUC of 0.7886 and with sensitivity of 63.5% and specificity of 89.2% (Fig. 1b).

Predicted by multiple GEE result, we also obtained Infection Predict Score:

$$\text{Infection Predict Score} = 0.4193 + 0.0782 \cdot \text{SDI} + 0.0997 \cdot \text{fever temperature} + 0.0341 \cdot \text{CRP} + 0.0005 \cdot \text{PCT} - 0.0006 \cdot \text{Lymphocyte percentage} + 0.0005 \cdot \text{NLR} - 0.0125 \cdot \text{Hb} + 0.0086 \cdot \text{SLEDAI 2K renal score}.$$

When cut at a value of 0.58286, there will be the largest Youden Index; that is, when the Infection Predict Score is greater than 0.58286, it will be classified as acute infection; if it is less than 0.58286, it will be classified as without acute infection.

Development of a calculator model to simultaneously differentiate flares from infections

Multinomial logistic regression which described the probability of being in a specific group was used to analyze the individual effects of covariates (independent variables) on the discrete nominal outcomes. We select a total of 10 variables (SDI, SLEDAI 2K, fever temperature, PCT, lymphocyte percentage, NLR, Hb, PLT, RPR, C3) to establish multinomial logistic regression. The regression formula obtained by multinomial logistic regression is as follows:

1. $\ln(\pi_A/\pi_D) = -11.932 - 1.3896 \times \text{DamgeIndex} + 0.4166 \times \text{SLEDAI} - 2K + 9.9529 \times \text{fever temperature} + 36.4342 \times \text{PCT} + 1.028 \times \text{lymphocyte percentage} + 1.3915 \times \text{NLR} - 1.0061 \times \text{Hb} - 0.00784 \times \text{PLT} - 21.2367 \times \text{RPR} - 0.0564 \times \text{C3}$
2. $\ln(\pi_B/\pi_D) = -6.1895 - 1.5815 \times \text{DamgeIndex} + 0.2968 \times \text{SLEDAI} - 2K + 10.9273 \times \text{fever temperature} + 36.1573 \times \text{PCT} + 0.9752 \times \text{lymphocyte percentage} + 1.3517 \times \text{NLR} - 0.9872 \times \text{Hb} - 0.0229 \times \text{PLT} - 56.2559 \times \text{RPR} - 0.0434 \times \text{C3}$
3. $\ln(\pi_C/\pi_D) = -12.5014 - 1.7698 \times \text{DamgeIndex} + 0.3917 \times \text{SLEDAI} - 2K + 10.0168 \times \text{fever temperature} + 35.6598 \times \text{PCT} + 1.0357 \times \text{lymphocyte percentage} + 1.3925 \times \text{NLR} - 0.8858 \times \text{Hb} - 0.00679 \times \text{PLT} - 18.9057 \times \text{RPR} - 0.0646 \times \text{C3}$

By inputting the value of the selected parameters into these three equations, we can get the ratios value of π_A , π_B , π_C and π_D , respectively. If the value obtained is larger, the probability of being classified into that nominal group is greater (the group divided into D is the reference group), then we will classify particular episode into that group (group A, B, C). That is, if calculated $\ln(\pi_A/\pi_D)$ was greater than 1 and was the biggest number compared with others ($\ln(\pi_B/\pi_D)$ and $\ln(\pi_C/\pi_D)$), the patient was categorized as group A. If all three calculated numbers [$\ln(\pi_A/\pi_D)$, $\ln(\pi_B/\pi_D)$, and $\ln(\pi_C/\pi_D)$] were below 1, the patient was categorized as group D. With combination of these ten parameters, we can simultaneously predict four groups with accuracy of 70.13% for infected-active group, 10% for infected-inactive group, 59.57% for noninfected-active group, and 84.62% for noninfected-inactive group, respectively. From our multinomial logistic regression analysis, we identified SDI, SLEDAI 2K, fever temperature, PCT, lymphocyte percentage, NLR, Hb, PLT, RPR, and C3 as influencing factors for simultaneously differentiating activity flares from acute infections.

Evaluation of possible associated interaction between acute infection and activity flare

In order to observe whether there is an associated interaction between acute infection and activity flare, we intend to compare parameters from combined groups with or without infection (Fig. 2). We found that the levels of CRP, PCT, lymphocyte percentage, NLR, PLR, SLEDAI 2K and SDI from combined group with infection were significantly higher than those of combined groups without infection. But the levels of ESR, C3 and C4 are not significantly different between the two combined groups. Our results (elevated levels of SLEDAI 2K, SDI, NLR, and PLR under noninfected condition) indicate that acute infections might play the

trigger role of activity flare. On the other hand, for those protein participate in both SLE disease inflammation and acute phase inflammation, there is no significant difference in their levels of ESR, C3 and C4 with or without regarding infections.

Trend analysis of parameters changes with time through hospitalization

The results of their mean baseline level and changes per time interval in different groups are shown in **supplement** Table 2. There significant differences for ESR, NLR, lymphocyte percentage, C3, C4, as shown in Fig. 3. From Fig. 3 (a) we found that ESR decreased with time, but the trend of decreasing was more prominent in group A than in group C. ESR appears to be a useful biomarker for SLE activity assessment. An elevated ESR is included in three out of five validated SLE activity scores [37]. The reported trend of ESR from Dima *et al.* are similar with our trend analysis indicating that the higher initial ESR level reflect the effect of both activity and infections in group A. From the trend difference between group A and C, we could differentiate noninfected-active SLE episodes (group C) from infected-active SLE episodes (group A) by the change patterns of ESR, NLR, lymphocyte percentage, C3, and C4 over time.

Discussion

In contrast to previous reports, the most important parameters used to establish the predictive model in our study consisted of four domains under simultaneous evaluation; *i.e.*, inflammation (CRP, ESR, PCT), hematology (WBC, PLT, Hb, lymphocyte percentage, NLR, PLR, RPR), the complement system (C3, C4), and clinical status (SLEDAI, SDI)(Fig. 4). This classification approach is conceptually close to the latest version of the SLE classification criteria [43]. Obtaining several parameters simultaneously remains necessary to differentiate flare from infection [32]. We hope to include more molecular biomarkers and genetic signatures in our model in the future, and the related mechanisms will be further explored.

This was a rare study focusing on distinguishing flare from infection in pediatric-onset SLE patients, and the study design closely approximated real-world clinical practice. Studies similar in design to ours have been reported previously [18, 20, 21, 28, 32]. Thus, our study design was similar to those for disease registries derived from the systematic collection of information from patients diagnosed with a particular disease (SLE). The management of pediatric SLE requires ongoing monitoring of patients, with the collection of data on many parameters/markers. The analysis of such markers allows us to anticipate the occurrence of clinical events and discriminate between flares and infections.

NLR and PLR have been used as prognostic indicators for malignancy and are associated with morbidity and mortality in chronic diseases [44]. Previous studies showed that NLR is associated with rheumatoid arthritis and psoriasis. Recently, higher NLR and PLR are both positively correlated with SLEDAI-2K score and disease activity [23, 27, 44]. On the other hand, NLR is associated with infection and has been used as an indicator of bacteremia [23, 24, 28]. Our result showed NLR were associated with both disease flare

and infection. In adult SLE cohorts, blood cell count ratios appeared to be more informative than blood cell counts *per se* because pancytopenia and thrombocytopenia tend to occur in SLE [45]. We observed the same tendency in our pediatric-onset SLE patients. Ideally, PLR values should be combined with values for NLR and other inflammatory markers to facilitate a more holistic determination [45]. Here, we demonstrated that the NLR and PLR are important complementary hematological indices that provide additional information about disease activity, presence of neutrophilic inflammation, infectious complications, disease severity, and organ damage in SLE.

The relationship between infections and SLE disease damage was difficult to evaluate. Infection itself was reported to both potentially facilitate or protect against the development of SLE [10, 46]. Results from a adult lupus cohort study in Latin America also showed that increased disease activity and damage accumulation were predictive of infection [39]. One study demonstrated that there is a positive correlation between SDI score and the number of recurrent major infections in pediatric-onset SLE [47]. Sit *et al.* also reported that disease damage was significantly associated with a greater number of episodes of major infection [48]. These findings are consistent with our study the associated between SDI with infection ($P < 0.0001$) by using multivariate GEE. Our results also indicated that the SDI and SLEDAI-2K renal scores could be used to accurately predict acute infection, consistent with previous reports.

The utility of traditional markers (e.g., CRP, ESR, PCT) for detecting infection in SLE patients has been discussed for several decades. CRP is an acute-phase reactant synthesized by the liver during IL-6 regulation and is known as an inflammatory biomarker [37]. CRP levels were reported to increase during infection, arthritis, and serositis in SLE patients [14]. One study showed that CRP level was more sensitive and specific in diagnosing bacterial infection compared to PCT level [41]. Immune complexes induce severe inflammation via conventional pro-inflammatory pathways, including those for cytokines such as TNF- α and IL-6 (which leads to CRP production). On the other hand, the same immune complexes induce the production of type I IFNs and various immunoregulatory cytokines. The simple consequence is reduced production of CRP in active SLE (group B > group A) despite increased IL-6 levels, which are visible in concomitant infection [18, 28, 32, 37]. PCT is a precursor peptide of calcitonin associated with invasive bacterial infections.

Normally produced by parafollicular C cells, PCT is released in response to bacterial toxins and IL-1 β [8]. PCT has good specificity for distinguishing acute bacterial infection from disease flare in patients with autoimmune diseases, regardless of steroid use. The mechanism underlying PCT production after inflammation and its role are still not completely understood [19]. Limited information is available regarding plasma PCT levels in patients with active SLE. Patients with active SLE may have slightly increased PCT levels [15, 18]. However, Garvand *et al.* noted an unusual phenomenon PCT levels were high during macrophage activation syndrome (MAS) episodes in lupus flares [49]. A systematic literature review suggested that PCT level and SLE disease activity are not correlated [15]. Our result of 4 groups comparison showed that the active disease group had higher PCT levels (group A > group B; group C > group D) regardless of the presence or absence of infection, suggesting that activity flare were associated with elevated PCT levels.

In the complement system, C3 and C4 have traditionally been used to assess SLE disease activity. However, studies of C3 and C4 consumption in SLE flares indicated that as markers, C3 and C4 exhibited low sensitivity and a wide range of specificity [12]. One reason for these inconsistent results may be because complement proteins participate in both autoimmune (SLE activity) and inflammation (infection) responses. As a consequence, inflammation due to infection increases, but immune complex consumption during disease activity decreases complement protein levels. Hence, the levels of C3 and C4 are regulated by both mechanisms [37]. Interestingly, decreases in C3 or C4 levels are not detected in some patients. Instead, the levels can increase relative to the baseline during flare episodes [50]. Our results in Fig. 3 showed that the C3 and C4 levels in group C were significantly increased with time may be related to this phenomenon.

Conclusions

Infections are a major cause of morbidity and mortality in SLE patients. Infections might mimic and even trigger SLE flares. To distinguish acute infection from activity flare always remains a clinical challenge. The proposed approach (Activity predict score, Infection predict score, and multinomial logistic regression formula) could differentiate flares from infections in pediatric SLE patients. Clinicians could make appropriate judgement and treatment decisions based on the combination of parameters from four different domains simultaneously, including inflammation (CRP, ESR, PCT), hematology (Lymphocyte percentage, NLR, PLR), complement (C3, C4), and clinical status (SLEDAI, SDI) in daily clinical practice (Fig. 4).

Limitations

This study was preliminary and was limited by that there were only 50 different patients with 185 episodes. Repeated measures in a single patient may introduces potential bias. More molecular biomarkers and genetic signatures should be included and evaluated in our model to explore related underlying mechanisms. Because autoantibodies are not routine laboratory inspections, so that autoantibody profiles are included in missing data due to insufficient data quantities, which may have some impact on our evaluation results.

Abbreviations

AR: autoregressive; CRP:C-reactive protein; ESR:erythrocyte sedimentation rate; GEE:generalized estimating equation; NLR:neutrophil-to-lymphocyte ratio; PCT:procalcitonin; PLR:platelet-to-lymphocyte ratio; RPR:RDW-to-platelet ratio; SLE:Systemic lupus erythematosus; SLEDAI:Systemic Lupus Erythematosus Disease Activity Index; SDI:Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Declaration

Ethics approval and consent to participate

Attached file (IRB approval-201505105RINC-English)

Consent for publication

Not applicable

Availability of data and materials

1. All data generated or analysed during this study are included in this published article [and its supplementary information files].
2. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LuoKL: the conception of the work, the acquisition, analysis, and interpretation of data and have drafted the work.

Yang YH, Lin YT, Hu YC, Yu HH, Wang LC, and Chiang BL: the acquisition and analysis of data.

LeeJH: the conception and design of the work, the acquisition, analysis, and interpretation of data, have drafted the work and finally substantively revised it.

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Figures

Figure 1

Receiver Operating Characteristic (ROC) curves for prediction of (a) activity flares and (b) acute infections. In (a), ROC for SDI score, SLEDAI 2K score, NLR, Hb levels, platelet levels, RPR, and C3 levels according to univariate GEE result (dashed line), and their combination (multi flare GEE) according to multivariate GEE result (solid line) to predict activity flare is shown. The area under curve (AUC) were shown within parentheses. In (b), ROC for SDI score, fever temperature, CRP levels, PCT levels, lymphocyte percentage, NLR, Hb levels, and SLEDAI 2K renal score according to univariate GEE result (dashed line), and their combination (multi infection GEE) according to multivariate GEE result (solid line) to predict acute infection is shown. The area under curve (AUC) were shown within parentheses.

(a)

(b)

(c)

(d)

Figure 2

Comparison for mean values of major parameters from infected (infected-active plus infected-inactive; A+B) groups vs noninfected (noninfected-active plus noninfected-inactive; C+D) groups. Parameters from four different domains, including (a) inflammation (CRP, ESR, PCT), (b) hematology (NLR, PLR, lymphocyte percentage), (c) complement (C3, C4), and (d) clinical status (SLEDAI, SDI), are depicted.

Figure 3

The average changing levels with time of (a) ESR, (b) NLR, (c) lymphocyte percentage, (d) C3, and (e) C4 during hospitalization are shown from a subset of 24 patients accounting for 29 times of admission. The trend for NLR showed that the NLR in group C were significantly decreased with time while those of group A were significantly increased with time. For lymphocyte percentage trend, the trend in group C was gradually increased with time while those of group A was sharply decreased with time. From Figure (d) &

(e), we found that the C3 and C4 levels in group C were significantly increased with time while those of group A were significantly decreased with time.

Figure 4

Investigation scheme and brief summary of our study. When SLE patients encounter clinical indications, through the evaluation model based on the parameters of the four main domains, we can make a correct evaluation and appropriate treatment. CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; GEE: generalized estimating equation; NLR: neutrophil-to-lymphocyte ratio; PCT: procalcitonin; PLR: platelet-to-lymphocyte ratio; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

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