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Serum IL-18 as biomarker in predicting long-term renal outcome among pediatric-onset systemic lupus erythematosus patients

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Abstract

An urge of biomarker identification is needed to better monitor lupus nephritis (LN) disease activity, guide clinical treatment, and predict patient's long-term outcome. With the proinflammatory effect and its association with inflammasomes, the significance of interleukin-18 (IL-18) among pediatric-onset systemic lupus erythematous (pSLE) patient, especially, its importance in predicting long-term renal outcome was investigated.

In a pSLE cohort of 96 patients with an average follow-up period of 10.39 ± 3.31 years, clinical data and laboratory workups including serum IL-18 were collected at time of disease onset and 6 months after treatment despite their initial renal status. Through Cox regression analysis, the parameters at baseline and at 6 months posttreatment were carefully analyzed.

Average age of all cases was 12.74 ± 3.01 years old and 65 of them underwent renal biopsy at the time of diagnosis. Nine subjects (9.38%) progressed to end-stage renal disease (ESRD) and 2 cases (2.08%) died during follow-up. Through multivariate analysis, serum IL-18 level 6 months posttreatment was found to be the most unfavorable factor associating poor clinical outcome despite patient's initial renal status. In addition, the presentation of serum IL-18 in its correlation with SLE global disease activity as well as the presence and severity of LN were all significant ($P < 0.001$, $P = 0.03$, and $P = 0.02$, respectively). The histological classification of LN, however, was not associated with the level of IL-18 among the pSLE patients ($P = 0.64$).

The role of serum IL-18 as biomarker representing global disease activity and status of renal flares among pSLE population was shown for the first time. Additionally, we have identified IL-18 at 6 months posttreatment a novel marker for long-term renal outcome prediction.

Abbreviations: anti-dsDNA Ab = anti-double-stranded DNA antibody, C3 = complement 3, eGFR = estimated glomerular filtration rate, ESRD = end-stage renal disease, IL-18 = interleukin-18, LN = lupus nephritis, pSLE = pediatric-onset systemic lupus erythematosus, ROC = receiver-operating characteristic, SLE = systemic lupus erythematosus, SLEDAI = SLE disease activity index, Th1 = helper T cells, type 1, Th2 = helper T cells, type 2.

Keywords: biomarker, interleukin-18, lupus nephritis, systemic lupus erythematosus, treatment response

1. Introduction

Pediatric-onset systemic lupus erythematosus (pSLE) is an autoimmune disease with multiorgan involvement and accounts for 15% to 20% of all systemic lupus erythematosus (SLE) cases.[1] Although many of the clinical manifestations were similar with the adult onset form, lupus nephritis (LN) among the pediatric population has been suggested to differ from the adult onset cases for its abrupt onset, high prevalence, and relative poor response to current treatment regimen.[2–6] According to previous studies, as high as 50% to 78% of pSLE cases suffered renal damages,[2,4,7] and 18% to 50% of these cases subsequently progressed to end-stage renal disease (ESRD).[8–10] Additionally, WHO class IV diffuse proliferative glomerulonephritis, the subgroup known with the worst outcome, is the most common histopathological findings of LN among pSLE patients accounting for half (40%–55%) of the cases.[10–13] To date, invasive renal biopsy remains the gold standard in determining LN classification, directing therapeutic strategy and predicting treatment outcome. In hope to ease patient anxiety bypassing such invasive procedure, researchers have searched and characterized various serum and urine markers associating LN activity, histopathological classification, and treatment response.

Level of serum interleukin-18 (IL-18) and its associated binding protein (IL-18BP) have previously been shown to correlate the severity of various autoimmune diseases in clinical

Editor: Angelo Valerio Marzano.

CW and HY contributed equally to this work.

Authorship: CW and HY participated in the design of the study, contributed to the acquisition, analysis, and interpretation of data for the work and drafted the manuscript; HY and SL helped with the statistical analysis; TY, SL, and JH revised the work critically for important intellectual content; JH conceived of the study, participated in its design and coordination, and helped revised the manuscript; and all authors read and approved the final manuscript.

Funding/support: This study was funded by Chang-Gung Memorial Hospital (CMRPG3D1352).

The authors have no conflicts of interest to disclose.

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Medicine (2016) 95:40(e5037)

Received: 2 May 2016 / Received in final form: 30 August 2016 / Accepted: 9 September 2016

http://dx.doi.org/10.1097/MD.0000000000005037

settings as well as experimental models,[14–17] and several mechanisms have been postulated in attempt to explain these findings. First, IL-18 is an important proinflammatory cytokine. It induces IL-1, tumor necrosis factor-alpha, and chemokines synthesis; enhances costimulatory and adhesion molecules expression; and results in crucial player recruitment and inflammation initiation.[18] Second, when act in synergy with other cytokines, IL-18 is capable of activating natural killer cells and various helper T cells (such as helper T cells, type 1 [Th1], helper T cells, type 2 [Th2], and Th17) in producing interferon-gamma (IFN-γ), IL-4, IL-5, IL-17, and various mediators to instruct cell activation and promote the release of matrix metalloproteinases.[18–21] Third, IL-18 precursor is constitutively expressed in many cells.[22] Only upon caspase-1 catalyzation, however, that its precursors can be processed into an active, mature form for released.[23,24] Thus, the elevation of IL-18 may in fact signal an increment caspase-1 activity in SLE, which has been demonstrated in various LN murine models contributing the development of autoimmune-related renal injury.[25]

Considering the potential pathological role of IL-18 in SLE, several studies have investigated the expression of IL-18 and its binding protein in SLE patient serum attempting to correlate its level with various SLE disease statuses since 2000.[15–17,26] To our knowledge, however, no study so far has looked into the association of serum IL-18 with LN specifically among the pediatric onset population or deliberated its value in long-term outcome prediction. Herein, we explored the level of IL-18 with SLE disease activity and renal performance among the pSLE population. In addition, we postulated that it also serve as a potent biomarker in predicting long-term renal outcome.

2. Material and methods

2.1. Subjects

Data of 118 pSLE patients who met the 1997 American College of Rheumatology revised criteria,[27,28] diagnosed between May 2005 and August 2011, were retrospectively recruited from the Pediatric Rheumatology Clinic at the Chang Gung Memorial Hospital in northern Taiwan. Patients with disease onset age <18, with serum samples available at time of diagnosis and 6 months following treatment were invited to participate this study regardless of their renal status. All subjects were regularly monitored for their clinical and laboratory parameters. Those who lack baseline information in our hospital, died within 6 months from disease onset, remained alive but had follow-up period shorter than 3 years, had diagnosis of mixed connective tissue disorder, or preexisting major organ disease such as complex congenital heart disease or chromosome anomaly were excluded. A written informed consent was collected from all the subjects participating the study and/or their legal guardian. The research was in compliance with the Declaration of Helsinki and the study design was approved by the local ethics committee (IRB No.: 103-1246A3).

2.2. Clinical information and laboratory tests

The patients were evaluated at time of pSLE diagnosis and every 2 weeks to 3 months for their clinical manifestations, laboratory tests, and disease activity indices. Complement level was examined by nephelometry while anti-double-stranded DNA antibody (anti-dsDNA Ab) was measured by enzyme-linked immunosorbent assay. Complete blood cell counts, serum creatinine (Jaffe method), urinalysis (reflective photometry as well as microscopic examination), urinary protein, and creatinine were also collected. Estimated glomerular filtration rate (eGFR) was calculated by the MDRD equations:

$$\text{eGFR (mL/minute/1.73 m}^2) = 186 \times [\text{Serum Cre (mg/dL)}]^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ if female}).$$

Extra-renal SLE manifestations such as mucocutaneous manifestations (malar rash, discoid rash, oral ulcer, and photosensitivity), hematological disorders (hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia), serositis (pleuritis and pericarditis), nonerosive arthritis, central nerve system disorders (seizure, psychosis, organic brain syndrome, cranial nerve disorder, and lupus headache), and vasculitis were determined according to the 1997 American College of Rheumatology revised criteria[27,28] and SLE disease activity index (SLEDAI)-2K.[29]

2.3. Serum and urine collection

Patient serums were collected at time of pSLE diagnosis and 6 months after treatment. Samples from 47 gender and age match controls were also collected. Serum samples were obtained from peripheral blood in heparin tubes, centrifuged and stored at −80° C until use. Spot morning urine samples were collected along with each plasma sampling and centrifuged at 4000 rpm for 5 minutes at 4 °C to precipitate the sediments before they are stored at −80° C for further analysis.

2.4. Serum collection and measurement of IL-18 and IL-18BP

Serum concentration of IL-18 and IL-18BP were determined by sandwich enzyme-linked immunosorbent assay reagent kits obtained from R&D system (Minneapolis, MN). The assay was performed according to the manufacturer's instructions. The appropriate recombinant human protein was used to establish the standard curve for each assay, respectively. Free IL-18 level was calculated with the law of mass action as previously described by Migliorini et al.[16]

Urine IL-18 was measured by the Bio-Plex Pro RBM kidney toxicity assays (Bio-Rad Laboratories) using the Luminex's XMAP Technology based multianalyte suspension array. After serial process according to the manufacturer's protocol, the beads were drawn single file through a flow cell where they were excited by 2 lasers. Using a dual-laser-based reader, beads are analyzed for the detection antibody and the internal bead signature, identifying both the protein analyzed and the level bound to the bead. Urine creatinine level was also measured for urine IL-18 standardization.

2.5. Renal biopsy and renal histopathology

Renal biopsy was performed only on patients with evidence of renal involvement. This included persistent hematuria, proteinuria (daily urinary protein excretion ≥500 mg/day or at least 1+ on urinalysis), cellular casts, and the presence of hypertension, unexplained abnormal serum creatinine level, or glomerular filtration rate ≤90 mL/min/1.73 m^2.[27]

Renal biopsy specimens were fixed in formaldehyde for light microscopy, direct immunofluorescence examination, and electron microscopy. Histological classifications of LN were examined by certified pathologists according to the World Health Organization (WHO) and the International Society of Nephrology and the Renal Pathology Society (ISN/RPS) systems.[30,31]

2.6. Treatments

The treatment protocol for LN class III and IV was based on the National Institutes of Health (NIH) protocol as previously described.[32–34] In brief, the patients received monthly intravenous cyclophosphamide (ivCYC, 0.5–1 g/m^2 body surface) pulse therapy for 6 months and/or intravenous methylprednisolone (ivMP, 30 mg/kg/dose) initial pulse therapy, followed by quarterly pulse therapy of ivCYC for another 6 doses as maintenance therapy. Oral prednisolone was also prescribed at an initial dose of 1 to 2 mg/kg/day and then at a maintenance dose of 2.5 to 10 mg/day. In addition, mycophenolate mofetil (MMF; dosage: 1 g/m^2/day divided twice daily) or azathioprine (2–3 mg/kg/day) was used in some patients as either induction or maintenance therapy instead of ivCYC. For those without LN at time of enrollment and those suffered from class I, II, V, and unclassified LN, oral prednisolone, azathioprine, and hydroxychloroquine were the drugs of choice. The overall treatments were comparable with worldwide standard practice.[35,36]

2.7. SLEDAI, renal SLEDAI, and treatment responses

The SLEDAI used was referenced from SLEDAI-2K published in 2002.[29] It is a weighted, cumulative index of lupus disease activity with a total score between 0 and 105. A higher score represented an increased disease activity. Renal SLEDAI consists of the 4 kidney-related criteria of the SLEDAI (i.e., hematuria, pyuria, proteinuria, and urinary casts). The presence of each 1 of these 4 parameters yields a score of 4 points, thus, the renal SLEDAI score can range from 0 to a maximal score of 16. The primary outcomes were ESRD or death and response status modified from previous studies,[35,37] categorized as complete remission (CR), partial remission (PR), no remission (NR), and renal flare were summarized in detail in Table 1. In addition, renal survival was defined as patient survival without ESRD.

2.8. Statistical analysis

Continuous data were summarized as means ± SDs and compared by an unpaired *t* test, paired *t* test, or the Mann–Whitney *U* test. Categorical data were expressed as number of patients and percentages and compared by Fisher exact test and one-way ANOVA. Predictors for poor outcome (ESRD or death) were evaluated by univariate Cox logistic regression, and statistically significant ($P < 0.05$) serum variables identified by univariate analysis were included in the multivariate analysis by applying multiple logistic forward Cox regression analysis. Receiver-operating characteristic (ROC) curve was used to explore the discrimination between those with poor outcome (ESRD or death) and to find the cutoff point for serum IL-18. The cutoff points were calculated by obtaining the best Youden index (sensitivity + specificity − 1).[38] Survival curves were draw using the Kaplan–Meier method and the difference between variables were estimated by Mantel–Cox test. For statistical analysis, 95% confidence intervals (CIs) were given. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using Stata version 11.1 (StataCorp., TX).

3. Results

3.1. Demographic, baseline characteristics, and clinical outcome

Ninety-six pSLE patients including 65 with and 31 without LN at time of SLE diagnosis were enrolled in this study after filtrated by the exclusion criteria, as shown in Fig. 1. There were 87 female and 9 male patients and the mean age of overall enrolled pSLE patients at time of diagnosis was 12.74 ± 3.01 years (range, 4.07–14.80 years), respectively. The average follow-up period was 10.39 ± 3.31 years (range, 3.92–14.82 years). At the end of the study period, 9 subjects (9.38%) progressed to ESRD and 2 cases (2.08%) died. Of the patients enrolled, 65 with LN had concomitant kidney biopsy performed at the time pSLE diagnosis and 42 (64.61%) of them suffered from class III or IV lesions. Five of the biopsied cases were grouped as uncategorized LN due to inadequate sampling (n = 2), undetermined histology (n = 2), and class III/V mixed pathology finding (n = 1).

The demographic data between those with and without LN were similar, as shown in Table 2. Patients with LN at time of diagnosis had significantly greater serum creatinine, overall disease activity, and urine protein/urine creatinine ratio, while those without had higher level of complement 3 (C3), hemoglobin, serum albumin, anti-dsDNA Ab, and eGFR. Central nerve system lupus with neuropsychiatric manifestations, serositis, and vasculitis was more prevalent in LN group than those without renal involvement, but no statistically significant was reached (12.31% vs 6.14%, $P = 0.39$; 9.23% vs 0, $P = 0.08$, and 16.92% vs 6.14%, $P = 0.16$).

3.2. Association of IL-18 with SLE disease activity, lupus nephritis, and treatment responses

As As shown in Fig. 2, level of serum IL-18 was higher among cases with pSLE regardless of their renal condition and when compared to healthy controls (849.20 ± 110.71 and 481.92 ± 83.18 vs 151.71 ± 120.95, both $P < 0.01$). In addition, it positively associated SLE disease activity ($r^2 = 0.13$; $P < 0.001$), elevated in the presence of LN ($P = 0.03$), and raised as renal SLEDAI increased ($P = 0.02$) at the time of SLE diagnosis. LN histological classification, on the other

Table 1

Definition of treatment outcome in the study.

Definition of treatment outcome
CR – GFR > 90 mL/min/m^2, no hematuria, no urine cast, no leukocyturia, and urine protein/urine creatinine ratio [U_p/U_{Cr}] < 0.2 or proteinuria < 200 mg/day
PR – at least 25% increase in GFR if abnormal baseline GFR or stabilization of previously normal GFR, U_p/U_{Cr} to a value of 0.2–2 or less than 1+ on urinalysis, inactive urinary sediment as CR required
NR – response not qualified for CR or PR
ESRD – stage V chronic renal disease: GFR < 15 mL/min/m^2 over 3 months, or either the need for renal transplantation or the long-term dialysis over 3 months
Renal flare (flare) – includes nephritic flares: recurrence of persistent hematuria or cast after remission; and proteinuric flares: persistent 50% increase in daily urinary protein (or recurrence of protein >2+ on urinalysis in 2 separate tests) after CR or PR

CR = complete remission, GFR = glomerular filtration rate ESRD = end-stage renal diseases, NR = no remission, PR = partial remission, SLE = systemic lupus erythematous

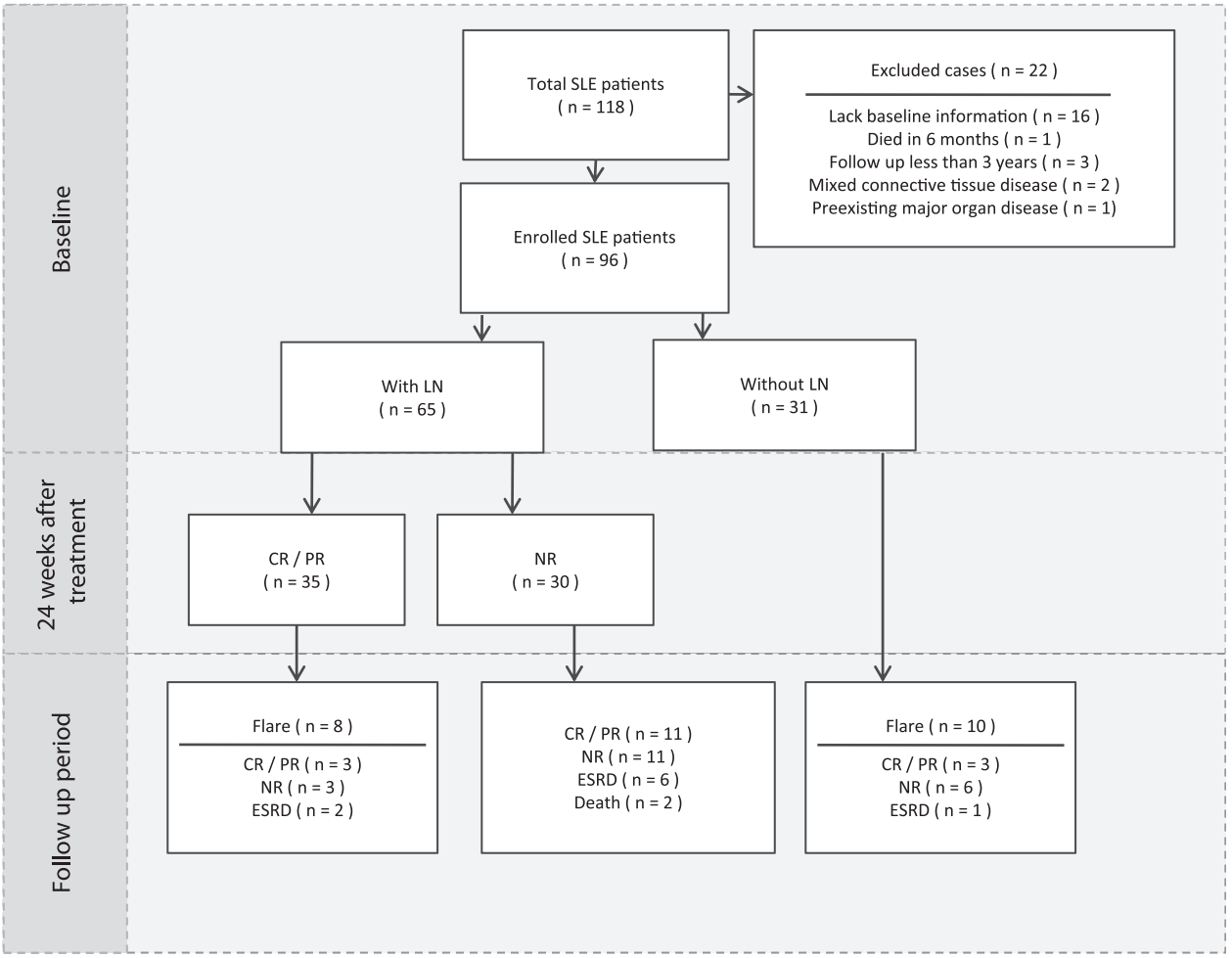

Figure 1. Flow chart with overview of patient's response to therapy. CR = complete remission, ESRD = end-stage renal diseases, LN = lupus nephritis, NR = no remission, PR = partial remission, SLE = systemic lupus erythematous.

hand, showed no correlation with concurrent IL-18 level at the time of diagnosis in this study ($P = 0.64$).

For cases with LN at baseline, levels of serum creatinine, anti-dsDNA Ab, and IL-18 declined significantly, while C3, complement 4 (C4), and serum albumin incremented 6 months after treatment (all $P < 0.001$). Among the serum markers, however, only serum IL-18 showed a slight difference in its level change between the groups responded (CR and PR) and those unresponded followed the initial 6 months of treatment (Δ IL-18 in group responded vs nonresponded: -628.70 ± 812.63 vs -248.32 ± 645.42, $P = 0.047$), as shown in Fig. 3.

3.3. Association of serum IL-18, IL-18BP, free IL-18, and urine IL-18 with lupus nephritis

The binding of IL-18BP with IL-18 had been reported previously to considerably alter cytokine's biological activity. Urine IL-18, on the other hand, was considered a marker for acute kidney injury but its role in associating LN has not yet been clearly analyzed. To further clarify the importance of IL-18BP, free form IL-18 and urine IL-18 in their association with lupus-related renal inflammations, we analyzed the association between the cytokines and compared the level of the listed proteins in the presence and absence of LN as summarized in Table 3.

The level of IL-18BP and free IL-18 but not urine IL-18 significantly associated serum IL-18 ($P < 0.001$, < 0.001 and 0.431, respectively. Data not shown). Only the level of serum IL-18 but not IL-18BP, free IL-18 or urine IL-18, however, reflected the activity of renal inflammation among the pSLE population ($P = 0.033$, 0.192, 0.361, and 0.605, respectively). We thus focus on the level of serum IL-18 together with other serum markers to evaluate the odds ratio in predicting long-term renal survival in the following study.

3.4. Analysis of serum markers in predicting long-term renal survival

The renal survival rate (characterized by survival without ESRD) in this entire study was 94.79% and 88.54% at 5 and 10 years. For those with LN at baseline, the 5- and 10-year patient renal survival rates were 92.31% and 84.62%, respectively.

Predictors and risk factors for poor outcome (death or ESRD) were evaluated among all pSLE cases and those with LN at baseline by Cox regression model as summarized in Table 4. Serum creatinine level and eGFR at baseline, as well as SLEDAI, renal SLEDAI, serum creatinine, IL-18, and anti-dsDNA Ab 6 months after treatment were factors influencing the outcome for all enrolled patients. By multivariate analysis, the strongest risk

Table 2

Characteristics of study subjects at time of enrolment.

Characteristics	LN		P
	With (n = 65)	Without (n = 31)	
Age, year	12.56 ± 3.05	13.10 ± 2.94	NS (0.42)
Sex (% female)	58 (85.96)	29 (93.10)	NS (0.39)
Duration of follow-up, year	10.42 ± 3.20	9.40 ± 3.41	NS (0.16)
SLEDAI score (mean ± SD)	18.77 ± 8.22	6.71 ± 4.46	<0.001
Renal SLEDAI (mean ± SD)	7.69 ± 3.63	0.39 ± 1.20	<0.001
anti-dsDNA Ab, IU/mL	428.05 ± 365.38	145.51 ± 117.60	<0.001
C3, mg/dL	57.52 ± 44.83	78.30 ± 30.34	0.02
C4, mg/dL	8.84 ± 6.15	11.52 ± 7.96	NS (0.07)
WBC, ×1000 cells/mm	7.99 ± 5.13	6.19 ± 2.91	NS (0.08)
Hemoglobin, g/dL	10.40 ± 2.20	11.59 ± 3.98	0.01
Platelet, /mm^3	193.52 ± 193.62	114.50 ± 130.83	NS (0.22)
Serum albumin, g/dL	3.26 ± 8.34	4.10 ± 0.47	<0.001
Serum creatinine, mg/dL	0.75 ± 0.30	0.63 ± 0.31	0.03
eGFR, mL/min/1.73 m^2	139.40 ± 77.11	140.70 ± 36.07	NS (0.93)
Urine protein/urine creatinine	2.40 ± 5.55	0.35 ± 0.56	<0.001
WHO class of LN (n%)			
I	1 (1.54)	–	
II	13 (20)	–	
III	6 (9.23)	–	
IV	36 (55.38)	–	
V	4 (6.15)	–	
VI	0 (0)	–	
UC*	5 (7.69)	–	
Extra-renal manifestations (n%)			
CNS lupus	8 (12.31)	2 (6.14)	NS (0.39)
Serositis	6 (9.23)	0 (0)	NS (0.08)
Hematology	35 (53.85)	15 (48.39)	NS (0.74)
Arthritis	22 (33.85)	7 (22.58)	NS (0.27)
Mucocutaneous	38 (58.47)	17 (54.84)	NS (0.74)
Vasculitis	11 (16.92)	2 (6.14)	NS (0.16)

Continuous variables are shown as mean ± SD; categorical variables as number (%). anti-dsDNA Ab = anti-double-stranded DNA antibody, C3 = complement 3, C4 = complement, CNS = central nerve system, eGFR = estimated glomerular filtration rate, LN = lupus nephritis, NS = not significant, SD = standard deviation, SLEDAI = systemic lupus erythematous disease activity index, UC = un-categorized, WBC = white blood cell, WHO = World Health Organization.

* Includes 2 suboptimal samples, 2 undetermined histology, and 1 with mixed class III and V lesions.

factor among serum markers was the level of IL-18 at 6 months followed treatment (OR 1.265, $P = 0.015$). This was likewise for those with LN at baseline (OR 1.273, $P = 0.020$) as well.

3.5. Serum IL-18 as biomarker in predicting long-term LN outcome

ROC curves were used to explore discrimination between those with poor outcome (ESRD or death) and find the cutoff point for serum IL-18, as shown in Fig. 4. The areas under the ROC curve (95% CI) were 0.73 (0.58–0.89) and 0.73 (0.58–0.91) for all pSLE cases (data not shown) and those with LN at baseline, respectively. The highest combination of sensitivity and specificity were observed with cutoff levels of 241.0 pg/mL (81.82% and 61.90%) for all enrolled cases and 304.4 pg/mL (80.0% and 61.1%) for cases with LN at baseline in predicting poor outcome.

Comparisons of renal survival grouped by the level of serum IL-18 6 months after treatment suggested that IL-18 level posttreatment can be used to predict long-term renal outcome for those with LN at time of SLE diagnosis ($P = 0.010$) (Fig. 5). No differences in long-term renal survival were observed comparing other serum markers such as serum creatinine, C3, C4, anti-dsDNA Ab, and albumin at baseline or after treatment. In addition, IL-18 level at baseline was also insufficient in predicting long-term LN renal outcome.

4. Discussions

This present study is the first to investigate the clinical significance of IL-18 in the prediction of long-term renal outcome specifically among the pediatric onset SLE population. From a pediatric SLE cohort of exclusively Asian ethnicity, with an average follow-up period of 10.39 ± 3.31 years, we found that a high serum IL-18 level 6 months posttreatment to be the most unfavorable factor associating poor clinical outcome among pSLE patients with renal involvement. In addition, the presentation of serum IL-18 was similar to that of the adult onset cases in its correlation with SLE global disease activity as well as the presence and severity of LN. The histological classification of LN, however, was not associated with the level of IL-18 among the pSLE patients.

IL-18, an inflammation-related cytokine crucial in both innate defense reactions and in Th1 activation, is responsible for immune-mediated pathologies and had been known to contribute the pathogenesis of various autoimmune diseases.[14] Although its role in SLE, unlike in rheumatoid arthritis, in psoriasis or in inflammatory bowel diseases, was less emphasized, a correlation of IL-18 with SLE disease activity was identified by Wong et al in 2000.[26] Later in 2001, Esfandiari et al[39] were able to reproduce lupus like glomerulonephritis, vasculitis, and skin lesions in SLE prone MRL/lpr murine model via daily IL-18 injection. With its potential pathogenic impact in SLE, the association of IL-18 with

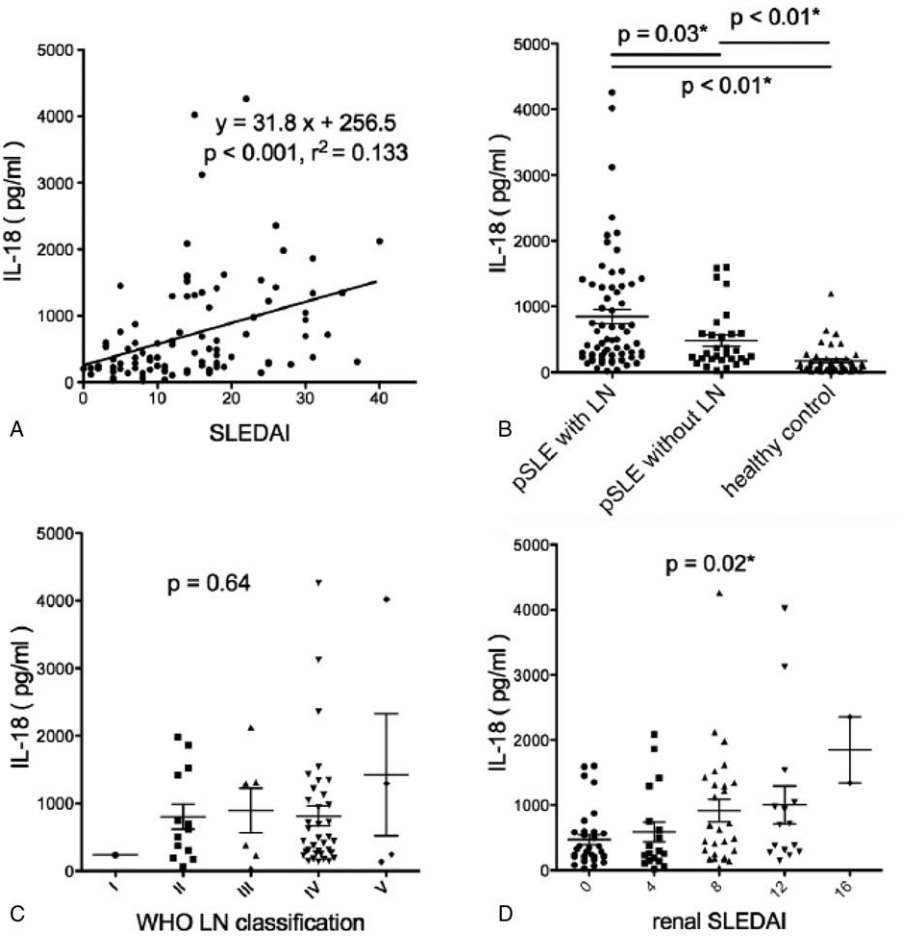

Figure 2. Association of IL-18 with SLE disease activity, LN activity, and renal histological classification. Dot plots depicting baseline serum IL-18 level (A) with SLE disease activity; (B) among normal controls and SLE cases with and without the presence of LN; (C) in different WHO LN histological classifications; and (D) with renal SLEDAI at time of diagnosis. Linear regression, Student *t* test, and one way-ANOVA were used for analysis and data were displayed as mean ± SEM. *P*-value ≤ 0.05 were considered significant. *Indicated *P*-value ≤ 0.05. ANOVA = analysis of variance, IL-18 = interleukin 18, LN = lupus nephritis, SEM = standard error of mean, SLE = systemic lupus erythematous, SLEDAI = SLE disease activity index, WHO = World Health Organization.

SLE disease activity has gained much attention since.[40,41] In 2002, Wong et al[42] documented a raise of serum IL-18 in cases with lupus-related renal manifestation and Calvani et al[43] later found that aside from patient serum, the expression of IL-18 was also increased within the glomeruli of nephritic patients specifically in the mesangial matrix and the infiltrating mononuclear cells.[43,44] Although the exact role of IL-18 in LN remained unknown, repeated precursor IL-18 cDNA vaccination and sequential generation of neutralizing IL-18 antibody has been documented to protect murine model from immune-related kidney damage.[45] Furthermore, several studies have also demonstrated the imbalance of Th1/Th2 immunity and the promotion of Th1 immune response as the pathogenesis behind LN development.[43,46] In fact, aside from the cytokine IL-18 itself, its natural antagonist, IL-18 binding protein, was also notice as a severity marker as well as a potential therapeutic target for LN.[15,47]

On the other hand, as a member of the IL-1 cytokine superfamily, IL-18 was produced as an inactive precursor and required further cleavage by the endoprotease, caspase-1, to generate a biologically active mature cytokine.[23,48] Inflammasome, the caspase-1-activation-plateform, essential for IL-18 production, was recently brought to attention in the pathogenesis of SLE.[25] Evidence showed that the polymorphisms in inflammasome genes are involved in the predisposition to systemic lupus erthematosus.[49] Activation of the NLRP3 inflammasome by neutrophil extracellular traps and LL-37 was enhanced in lupus macrophages.[50] Further, immune complexes formed by lupus-associated autoantigens, dsDNA and nuclear ribonucleoprotein, and their respective autoantibodies can activate the inflammasome machinery in monocytes.[51,52] Recently, Zhao, Tsai, and Yuan reported that inhibiting NLRP3 inflammasome by P2X7 antagonist, chemical compound, epigallocatechin-3-gallate, or isoflurane, the progression of LN in SLE murine model could be attenuated.[53–56] Similar findings were also demonstrated in caspase-1 knockout and pristane induced murine lupus models by Kahlenberg et al.[57] Despite the growing evidences among murine models, the role of inflammasomes in human SLE remained largely under investigated. Recently, Yang et al[58] analyzed the expression of NLRP3/NLRP1 inflammasomes in the peripheral blood mononuclear cells of SLE patients and Yang et al[59] demonstrated that NLRP3 inflammasome to be hyper-activated in macrophages among SLE patients.[59] Even though we did not look into the

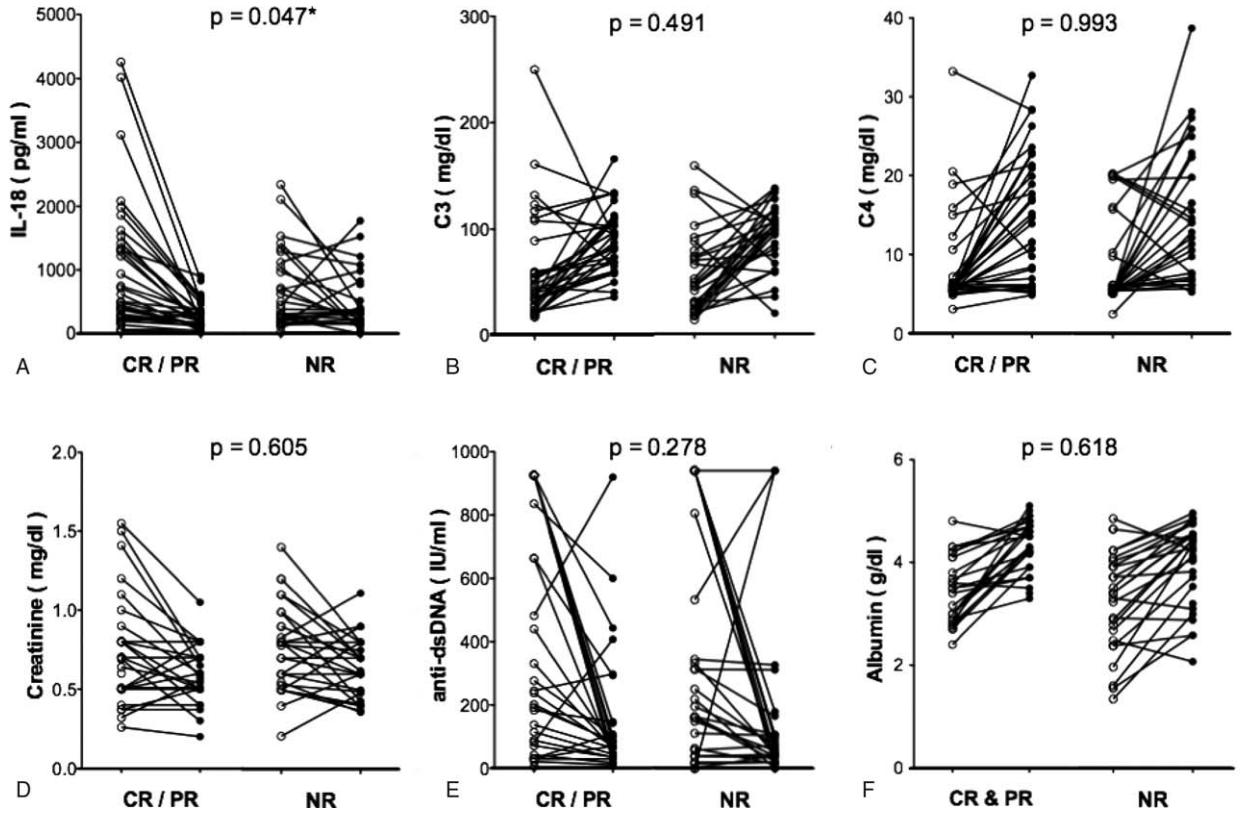

Figure 3. Serum markers associated with LN treatment response. Paired dot plots depicting serum levels of (A) IL-18; (B) C3; (C) C4; (D) creatinine; (E) anti-dsDNA, and (F) albumin at baseline (open circle) and 24 weeks after treatment (solid circle) in groups with (complete remission and partial remission) and without (no remission) treatment response. Student *t* test was used for analysis. *P*-value $\leq$0.05 were considered significant. *Indicates *P*-value $\leq$0.05. anti-dsDNA Ab = anti-double-stranded DNA antibody, C3 = complement 3, C4 = complement, CR = complete remission, IL-18 = interleukin 18, NR = no remission, PR = partial remission.

engagement of inflammasomes directly in the present study, a persisted high IL-18 level may potentially serve as a surrogate maker illustrating a hyper-inflammatory status apart from its recognized role in rendering the adaptive immune response.

With the proinflammatory nature of IL-18 and its importance in chronic inflammation regulating both innate and adaptive immune responses,[60] high level of serum IL-18 posttreatment may be considered as a symbol for ongoing inflammation that was not properly controlled by the regimen. Indeed, from the Cox regression model shown in Table 4, we notice that anti-dsDNA Ab, another serum marker sensitive to the fluctuation of disease activity and the status of inflammation,[61,62] also elevated among those with poor clinical outcome despite 6 months of treatment. Additionally, high SLEDAI, particularly high renal SLEDAI, illustrating a poor response to the management was likewise noticed to associate SLE patient's long-term outcome in the present study. Although the idea of using proinflammatory cytokine, IL-18, as biomarker to predict long-term prognosis was first introduced, complementary reports were published by Wu et al[34] and Houssiau et al stating that the most significant favorable factor was the achievement of early response within 6 month after treatment apart from patient's baseline renal condition.

Table 3

Serum and urinary levels of IL-18 and serum IL-18 BP in pediatric systemic lupus erythematous patients with and without nephritis.

Measurements				
Sample	**Proteins**	**With LN**	**Without LN**	***P***
Serum	Case no.	n = 65	n = 31	
	IL-18, pg/mL	849.20 ± 110.71	481.92 ± 83.18	0.033*
	IL-18 BP, pg/mL	5932.05 ± 5401.34	3642.03 ± 2808.58	0.192
	Free IL-18, pg/mL	679.36 ± 365.76	385.51 ± 461.04	0.361
Urine	Case no.	n = 65	n = 29	
	IL-18, pg/mL	6.81 ± 10.84	7.99 ± 13.29	0.605
	IL-18/Ucre ($\times 10^{-9}$)	11.26 ± 20.73	16.41 ± 32.41	0.272

Variables were shown as mean ± SD; *P*-value <0.05 were considered significant. BP = blood pressure, IL-18 = interleukin 18, IL-18BP = interleukin 18 binding protein, LN = lupus nephritis, SD = standard deviation, Ucre = urine creatinine.

Table 4

Predictors for poor outcome (death or ESRD) by Cox regression model.

A. All enrolled cases with pediatric onset systemic lupus erythematous

Parameters	Baseline		6 months after treatment	
Univariate logistic regression	**Odds ratio**	***P***	**Odds ratio**	***P***
Age	1.085 (0.873–1.349)	0.463	1.108 (0.855–1.386)	0.371
Gender (male)	4.179 (0.900–19.410)	0.068	4.179 (0.900–19.410)	0.068
SLEDAI	1.044 (0.978–1.115)	0.196	1.141 (1.006–1.293)	0.039*
Renal SLEDAI	1.112 (0.970–1.274)	0.127	1.286 (1.089–1.518)	0.003*
C3	0.993 (0.976–1.010)	0.427	0.981 (0.959–1.004)	0.106
C4	0.984 (0.891–1.087)	0.755	1.009 (0.934–1.089)	0.828
Anti-dsDNA Ab	1.001 (0.999–1.003)	0.207	1.002 (1.000–1.004)	0.033*
Serum IL-18	1.049 (0.984–1.117)	0.141	1.233 (1.064–1.428)	0.005*
Serum albumin	0.713 (0.333–1.526)	0.383	1.172 (0.806–1.705)	0.407
Serum creatinine†	1.270 (1.022–1.577)	0.031*	1.754 (1.130–2.723)	0.012*
eGFR	0.982 (0.964–0.999)	0.040*	0.987 (0.970–1.004)	0.146
Multivariate logistic regression				
eGFR	0.944 (0.875–1.018)	0.136	–	–
Serum IL-18	–	–	1.265 (1.047–1.527)	0.015*
Anti-dsDNA Ab	–	–	1.001 (0.998–1.004)	0.454
Serum creatinine†	0.607 (0.247–1.490)	0.276	1.515 (0.874–2.626)	0.139

B. Pediatric onset systemic lupus erythematous cases with lupus nephritis at time of diagnosis

Parameters	Baseline		6 months after treatment	
Univariate logistic regression	**Odds ratio**	***P***	**Odds ratio**	***P***
Age	1.084 (0.858–1.369)	0.499	1.092 (0.859–1.389)	0.472
Gender (male)	4.286 (0.835–21.991)	0.081	4.286 (0.835–21.991)	0.081
SLEDAI	1.011 (0.932–1.097)	0.791	1.074 (0.934–1.235)	0.318
Renal SLEDAI	1.028 (0.853–1.241)	0.769	1.193 (0.996–1.429)	0.055
C3	0.998 (0.982–1.014)	0.826	0.981 (0.958–1.005)	0.118
C4	1.012 (0.910–1.125)	0.827	1.002 (0.925–1.085)	0.963
Anti-dsDNA Ab	1.000 (0.998–1.002)	0.666	1.002 (1.000–1.004)	0.073
Serum IL-18	1.040 (0.975–1.110)	0.232	1.256 (1.058–1.490)	0.009*
Serum albumin	0.963 (0.407–2.277)	0.931	1.148 (0.800–1.647)	0.455
Serum creatinine†	1.197 (0.961–1.491)	0.108	1.753 (1.113–2.761)	0.015*
eGFR	0.987 (0.971–1.003)	0.104	0.988 (0.971–1.005)	0.156
Multivariate logistic regression				
Serum IL-18	–	–	1.273 (1.039–1.559)	0.020*
Serum creatinine†	–	–	1.600 (0.915–2.798)	0.099

anti-dsDNA Ab = anti-double-stranded DNA antibody, C3 = complement 3, C4 = complement, eGFR = estimated glomerular filtration rate, LN = lupus nephritis, SLEDAI = systemic lupus erythematous disease activity index.

† Odds ratio for serum creatinine was analyzed with an increment of 0.1 of mg/dL.

The importance of patients' underlining renal condition was not to be underscored in anticipating ultimate renal outcome on the other hand. Besides the unsatisfactory response to regimen and possibly the influence of inflammasome as previously discussed, serum creatinine level and kidney histological classification remain the leading factors directing pSLE patient's fate in the end. Seven out of the 36 cases (19.44%) with class IV nephritis eventually progressed to ESRD or death in the present study. This made diffuse glomerulonephritis the worst pathological finding for long-term renal survival, similar to what have previously been observed.[34,63,64] Furthermore, a higher baseline creatinine level and an elevated level of serum creatinine 6 months after treatment were documented by Houssiau et al[65] and us to correlate renal outcome, again addressing the denotation of underlining renal status in the overall prognosis of SLE patients.

Finally, differences between adult onset SLE and pSLE, specifically their renal manifestations, have been realized and discussed.[2,4,66] Compared with its adult onset form, SLE onset during childhood carried a higher risk of developing LN and a less response to therapy.[4,6,66] Although the histological classes of LN and initial renal manifestations are similarly distributed among the 2,[67,68] an increased number of SLE-susceptibility risk alleles and cytokines production, particularly involving type I interferon signaling, were associated with those with early onset.[69] Additionally, because interferon-alpha and IL-18 were noted to exert opposite regulatory effects on the IFN-γ production in macrophages regulating its inflammatory response,[70] it became interesting to clarify if IL-18 reacted in a similar pattern among the pSLE patients with those later onset. We found that serum IL-18 correlated with SLE global disease activity and the presence and severity of LN similar to those adult onset cases,[26,42] while the histological classification not. Moreover, the level of IL-18 in this present study is about 2 to 3 times higher than those previously reported.[26,42] Without side-by-side comparison and standard laboratorial technique, unfortunately, it would be impossible to conclude a higher IL-18 activity among the pSLE cases based on what we have observed.

Several limitations were noted in the present study, however. As this paper recruited pSLE patients of a single ethnicity, from a single medical center, may detract from the broader significance of the findings reported herein. Also, though serum samples were promptly stored at −80 °C once acquired, possible degradation of

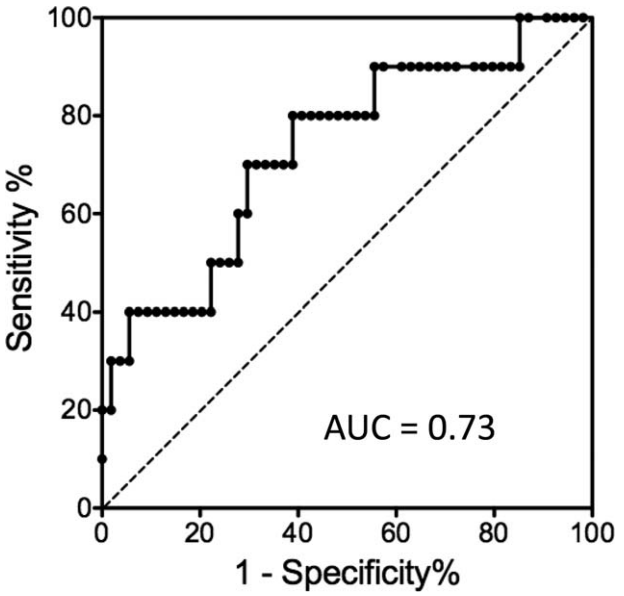

Figure 4. ROC curve of serum IL-18 for poor outcome (ESRD and death) among pediatric systemic lupus erythematous patients with LN at time of diagnosis. AUC = area under the ROC curve, ESRD = end-stage renal diseases, 18 = interleukin 18, LN = lupus nephritis, ROC = receiver-operating characteristic.

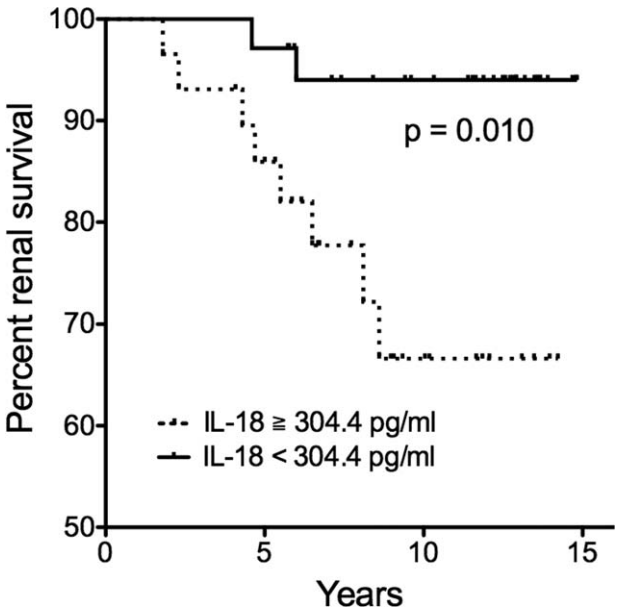

Figure 5. Kaplan–Meier survival curve for renal survival among pediatric systemic lupus erythematous patients with lupus nephritis at time of diagnosis. Log rank test was used for analysis. *P*-value ≤ 0.05 were considered significant. *Indicates *P*-value ≤ 0.05.

cytokine proteins during the years remained a factor to be considered. Longer follow-up period and accumulation of more patients is always beneficial. Nonetheless, further investigation on pathogenic mechanism of IL-18 and the potential role of inflammasomes in LN development may further improve the study.

5. Conclusions

In conclusion, our study among the pSLE patient not only echoed the role of serum IL-18 in SLE patient as a marker representing global disease activity, but also in renal flares, we expanded its utilization in prediction of the long-term renal outcome, suggesting an extending importance and a possible promising target for therapy advancement. Even though further investment is required to uphold our observation, through our thorough study, the importance of IL-18 in SLE pathogenesis is brought to discussion.

Acknowledgments

The authors thank Pi-Shuang Chu and Hsiu-Li Tsou for their contribution in data gathering and ELISA preparation. The authors also thank Chang-Gung Memorial Hospital for funding this study (CMRPG3D1352).

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