A Boy Presenting with a Fever and Pancytopenia Diagnosed with Systemic Lupus Erythematosus without a Positive Anti-ds-DNA Antibody Result or Hypocomplementemia: A Case Report

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Background: Hematological involvement, including anemia, leukopenia, lymphopenia, and thrombocytopenia, is one of the most common manifestations of childhood-onset systemic lupus erythematosus (cSLE). Specifically, relatively severe forms of hematological involvement, such as macrophage activation syndrome (MAS) and thrombotic microangiopathy, occur in the course of the disease. Positivity for anti-double stranded-DNA (ds-DNA) antibody and hypocomplementemia are important as not only criteria of diagnosing cSLE but also in the determination of the disease activity.
Case Report: A 13-year-old boy without pre-existing disease was referred to our hospital chiefly complaining of a fever for > 7 days, long-lasting malaise, nausea, and non-malar face rash. His blood examination showed pancytopenia and hyperferritinemia, but positive results for anti-ds-DNA antibody and hypocomplementemia were not recognized. Bone marrow aspiration revealed no evidence of malignant diseases, hemophagocytic lymphohistiocytosis, or MAS. A renal biopsy for the differential diagnosis of proteinuria and hematuria revealed class Ⅲa + V lupus nephritis, leading to the diagnosis of cSLE.
Conclusions: It is important for cSLE to be considered in patients with pancytopenia, even those without positive anti-ds-DNA antibody findings or hypocomplementemia, and for aggressive approaches to be adopted for the differential diagnosis, including a renal biopsy.

Key words: Anti-ds DNA antibody, Children, Hypocomplementemia, Pancytopenia, Systemic lupus erythematosus

INTRODUCTION

Childhood-onset systemic lupus erythematosus (cSLE) is a systemic autoimmune disorder characterized by multisystemic involvement and a chronic-relapsing course, with symptoms beginning before 18 years old. It accounts for approximately 10% of all SLE cases [1]. It is well known that cSLE often presents with a higher frequency of renal, hematological, and neuropsychiatric involvement as well as greater disease activity with life-threatening events than adult-onset SLE [2, 3].

Hematological involvement, including anemia, leukopenia, lymphopenia, and thrombocytopenia, is one of the most common manifestations of cSLE [4]. A nationwide survey of cSLE in Japan revealed that hematological involvement was recognized in 72.6% of patients at the SLE diagnosis and in 80.6% of those patients during the whole observation period, with hemolytic anemia specifically being noted in 12.4%, leukopenia in 52.2%, lymphopenia in 38.7%, and thrombocytopenia in 30.6% at the SLE diagnosis [5]. In particular, more severe forms of hematological involvement, such as macrophage activation syndrome (MAS) and thrombotic microangiopathy, also reportedly occur in the course of the disease and sometimes lead to a fatal outcome [6]. Thus, the differential diagnosis of hematological involvement in SLE is challenge, affecting the diagnosis and decision concerning treatment for individual SLE patients.

Regarding the classification criteria for SLE, positivity for anti-double stranded-DNA (ds-DNA) antibody and hypocomplementemia are important criteria for diagnosing cSLE. The Systemic Lupus International Collaborating Clinics (SLICC) group proposed hypocomplementemia, including low C3, C4, and CH50 levels, as an immunologic criteria in 2009 to improve the sensitivity of the SLE criteria [7]. In addition, because of the low diagnostic sensitivity (69%) for Japanese cSLE with the 1997 American College of Rheumatology (ACR) classification criteria [8], the diagnostic guidance was modified by a study group of the Japanese Ministry of Health and Welfare, with hypocomplementemia added to the ACR 1997 criteria, resulting in an improvement of sensitivity (77%) for Japanese cSLE [5]. The nationwide survey mentioned above also revealed that 91.9% of cSLE patients had a positive result for anti-ds-DNA antibody at the time of the diagnosis, and

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Table 1 Blood examination during the course of the disease

	On admission	At SLE diagnosis
White blood cell count (/μL)	1,500	800
Neutrophils (%)	61.0	52.0
Lymphocytes (%)	36.0	44.0
Monocytes (%)	2.0	4.0
Red blood cell count (/μL)	393×10^4	297×10^4
Hemoglobin (g/dL)	10.8	8.1
Hematocrit (%)	31.9	25.2
Platelets (/μL)	57×10^3	67×10^3
Aspartate aminotransferase (U/L)	49	85
Alanine aminotransferase (U/L)	35	80
Lactate dehydrogenase (U/L)	626	1,113
Erythrocyte sedimentation rate (mm/hr)	99	142
C-reactive protein (mg/dL)	0.019	0.01
Ferritin (ng/mL)	541	2,505
CH50 (U/mL)	45.4	38.5
C3 (mg/dL)	78	67
C4 (mg/dL)	19	19
Antinuclear antibody	1/80	1/80
(Pattern)	(Speckled)	(Speckled)
Anti-ds-DNA antibody (IU/mL)	< 10	< 10
Anti-Smith antibody (U/mL)	1.4 (-)	1.2 (-)
Anti-RNP antibody (U/mL)	< 2.0 (-)	< 2.0 (-)
Anti-SS-A/Ro antibody (U/mL)	> 1,200 (+)	> 1,200 (+)
Anti-SS-B/La antibody (U/mL)	2.0 (-)	1.5 (-)

ds-DNA, double-stranded DNA; SLE, systemic lupus erythematosus

96.2% had a positive result during whole observation period; hypocomplementemia was additionally noted in 78.0% and 88.2% of these patients, respectively [5]. These parameters are useful biomarkers for predicting disease activity during follow-up, especially in patients who develop lupus nephritis, and have a good predictive value for exacerbation [9, 10].

We herein report a difficult diagnostic case of a 13-year-old boy with a fever and pancytopenia whose renal biopsy revealed lupus nephritis, resulting in a diagnosis of cSLE despite negative for anti-ds-DNA antibody or hypocomplementemia.

CASE REPORT

The patient was a 13-year-old boy without pre-existing disease who was referred to our hospital with a chief complaint of a fever lasting over 7 days, long-lasting malaise, nausea, and a non-malar face rash. His blood examination revealed pancytopenia. He had no history of photohypersensitivity, oral ulcers, joint pains, dryness of the eyes or mouth, or decreased urine output. His family history was unremarkable.

On a physical examination, his body temperature was 37.9°C, blood pressure 93/57 mmHg, pulse rate 134 bpm, respiratory rate 20 bpm, and oxygen saturation 99%. A laboratory investigation at his first hospital visit revealed pancytopenia with white blood cell counts of 1,500/μL, hemoglobin 10.8 g/dL, and platelets 57,000/μL, as well as an elevated erythrocyte sedimentation rate of 99 mm/h and lactate dehydrogenase level of 626 U/L. His blood urea nitrogen, serum creatinine, and C-reactive protein values were within normal limits. His liver function test results were normal, and serum triglycerides and total cholesterol were not elevated (96 mg/dL, 149 mg/dL, respectively), but ferritin was elevated (541 ng/mL). His Coombs test was negative, with no evidence of hemolytic anemia, but the findings for anti-cardiolipin antibody, anti-β 2 glycoprotein-I antibody, and lupus anticoagulant did not meet the criteria for anti-phospholipid antibody syndrome (APS). Although antinuclear antibody (speckled pattern 1/80) and anti-SS-A/Ro antibody levels were elevated, other autoantibodies, including anti-ds-DNA antibody, anti-RNP antibody, anti-Smith antibody, and anti-SS-B/Lo antibody, were negative, and serum complement levels were not decreased (Table 1). His serum albumin was slightly decreased (2.9 g/dL), while urinalysis revealed proteinuria (protein/creatinine ratio of 2.0 in a single voided urine sample) and hematuria with epithelial and granular casts.

After his admission to our hospital, bone marrow aspiration revealed no evidence of malignant diseases, hemophagocytic lymphohistiocytosis (HLH), or MAS. Chest and abdominal computed tomography showed only hepatosplenomegaly. No abnormal findings were noted on magnetic resonance imaging of the head or cardiac ultrasonography.

The patient was also examined for Sjögren syndrome with the Schirmer test, Gam test, a labial biopsy for the minor salivary gland, and salivary MRI because of his high titer of anti-SS-A/Ro antibody. The Gam test and salivary MRI showed abnormal findings of Sjögren syndrome, resulting in a diagnosis of Sjögren syndrome. We were considering the possibility of Sjögren's syndrome that led to pancytopenia and proteinuria at this time.

Since his fever persisted after hospitalization with pancytopenia and hyperferritinemia was gradually progressing (Table 1), we performed a renal biopsy for

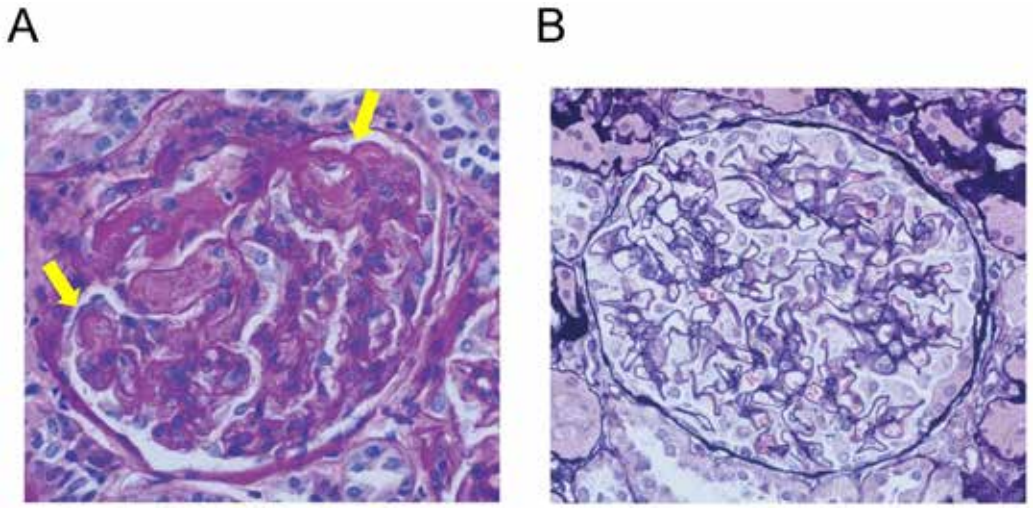

Fig. 1 The pathological characteristics of the renal biopsy in this patient. (A) The image for periodic acid-Schiff staining. Half of the 49 glomeruli showed segmental mesangial proliferation, and 1 showed a wire loop lesion (yellow arrow). Crescent and sclerosis were not recognized among the glomeruli. (B) The image for periodic acid-methenamine-silver stain.

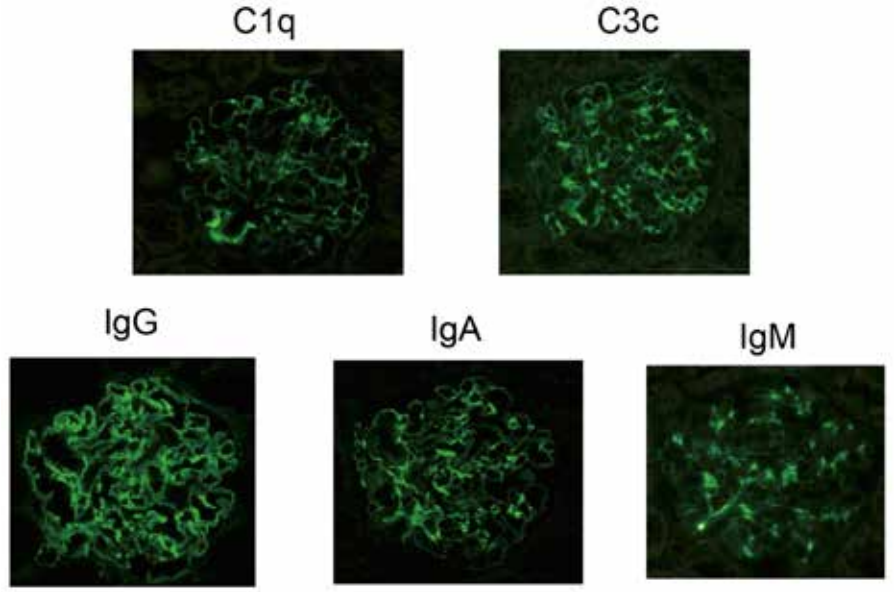

Fig. 2 The images for immunofluorescence staining revealed mesangial and peripheral deposits of immunoglobulin (Ig) G, IgA, IgM, C1q, and C3c.

a differential diagnosis concerning his urinary findings of proteinuria and hematuria. A light microscopic examination showed 49 glomeruli, among which half showed segmental mesangial proliferation, with 1 showing a wire loop lesion. Crescent and sclerosis were not recognized in those glomeruli (Fig. 1). An electron microscopic examination demonstrated spike formation in capillary basal lamina. An immunofluorescence examination revealed mesangial and peripheral deposits of immunoglobulin (Ig) G, IgA, IgM, C1q, and C3c, suggesting a full-house immunostaining pattern (Fig. 2). Based on these findings, we diagnosed him with class Ⅲa+V lupus nephritis according to the criteria proposed by the International Society of Nephrology/Renal Pathology Society (ISN/IRPS) [11]. Altogether, he was diagnosed with cSLE according to fulfillment of the presence of antinuclear antibody and lupus nephritis in the SLICC classification criteria [12], although anti-ds-DNA antibody and marked hypocomplementemia were not recognized at this time. He fulfilled only three criteria of ACR classification criteria including renal disorder, hematologic disorder, and ANA that did not meet the diagnosis of SLE at this time [8].

Initial treatment consisted of 3 courses of intravenous methylprednisolone pulse therapy (1,000 mg/day, consecutive 3 days, 1-week interval), followed by oral prednisolone (45 mg/day), and both hydroxychloroquine (200 mg or 400 mg on alternate days) and mycophenolate mofetil (1,000 mg/day) were administered, according to the Japanese pediatric guideline for the treatment of SLE [5]. After the first course of methylprednisolone pulse therapy, he immediately responded to treatment, which led to alleviation of fever and improvement of pancytopenia. His proteinuria accordingly decreased, and complete remission

Table 2 The sequential data of hemoglobin and erythrocyte sedimentation rate after treatment

	After 1st course of MPT therapy	After 2nd course of MPT therapy	After 3rd course of MPT therapy	At discharge
Hb (g/dL)	8.5	9.5	10.4	12.2
ESR (mm/hr)	78	75	26	30

ESR, erythrocyte sedimentation rate; Hb, hemoglobin; MPT, methylprednisolone pulse therapy

of proteinuria was achieved after the second course of methylprednisolone pulse therapy. Prednisolone was able to gradually be reduced to a low dose after three courses of methylprednisolone pulse therapy. However, since the ESR was still elevated regardless of improvement of anemia (Table 2), and both the anti-ds-DNA antibody and serum complement levels had remained normal since the disease onset, meaning they could not be used as biomarkers for the disease activity in this patient, careful follow-up was deemed necessary to maintain patient stable disease with immunosuppressive therapy.

DISCUSSION

In this patient who presented with a lasting fever, pancytopenia, hyperferritinemia, and proteinuria, a renal biopsy for the differential diagnosis of proteinuria and hematuria eventually revealed lupus nephritis, resulting in a diagnosis of cSLE without anti-ds-DNA antibody or hypocomplementemia, which are frequently recognized at the diagnosis of Japanese cSLE patients. Of note, those parameters were not altered due to disease activity during the disease course in this patient, but proteinuria and the ESR sensitively varied with disease activity. These characteristics might indicate the potential pathophysiology in cSLE patients without anti-ds-DNA antibody or hypocomplementemia, which help to improve clinical practice and thereby better diagnose and treat those patients.

Our patient presented with pancytopenia as an initial manifestation of cSLE. Furthermore, although progressing pancytopenia and hyperferritinemia were recognized, bone marrow aspiration revealed no specific findings, including activated macrophages with phagocytosis. Hematological involvement as an initial manifestation of SLE is not rare, but SLE patients with cytopenia tend to have a delayed diagnosis compared with non-hematological SLE [13]. In addition, pancytopenia was reportedly detected in 18.9% of cSLE [14] and 2.6%–9.8% of adult SLE patients [13, 15], suggesting that pancytopenia is a relatively rare hematological involvement at the diagnosis of SLE. There have been several studies including bone marrow findings in SLE, and several studies have showed dysplasia of erythroid, myeloid, and megakaryocyte in SLE patients' bone marrow, thus indicating that the bone marrow findings may reflect the disease severity, as well as bone marrow necrosis, increased reticulin fibrosis, dilated sinuses, and hypocellularity [16, 17]. In contrast, HLH and autoimmune myelofibrosis were reported as causes of cytopenia in SLE [6, 18]. Although our patient did not meet those disease criteria, it was deemed likely that pancytopenia and hyperferritinemia indicated a transition to HLH or MAS. Based on the present findings, pancytopenia in patients with dysplastic bone marrow but no malignant findings or activated macrophages might be considered symptoms of SLE.

The presence of anti-ds-DNA antibody was associated with strong diagnostic specificity and disease activity of SLE patients, so it is generally used to assess the efficacy of therapeutic approaches in clinical practice [19]. In fact, several studies have demonstrated that anti-ds-DNA antibody was significantly associated with active disease, including disease exacerbation and renal and hematological involvement, while a negative association was observed with central nervous involvement [19, 20]. Furthermore, the levels of anti-ds-DNA antibody had a significant correlation with disease activity [20]. However, in our case, anti-ds-DNA antibody was persistently negative during the course of the disease, making it difficult to evaluate the disease activity using this parameter. A previous study of SLE patients with and without anti-ds-DNA antibody showed that patients without anti-ds-DNA antibody had a higher prevalence of serositis than those with it but less frequently had renal involvement or a reduction in serum C4 levels [21].

In addition to anti-ds-DNA antibody, serum complement was also a useful marker for SLE patients. Similar to anti-ds-DNA antibody, hypocomplementemia was reportedly associated with a high prevalence of renal and hematological involvement and was correlated with the disease activity [22, 23]. Furthermore, hypocomplementemia had an association with APS-related features such as livedo reticularis, hemolytic anemia, and thrombocytopenia [24]. In particular, a low C3 level was associated with renal involvement and poor renal outcomes, while both low C3 and C4 levels were associated with stroke in the presence of lupus anticoagulant or anticardiolipin antibody [25]. Based on these observations concerning anti-ds-DNA antibody and hypocomplementemia, the presence or absence of anti-ds-DNA antibody and hypocomplementemia may indicate specific disease subsets in SLE, specifically with regard to renal and hematological involvement, and APS.

In these contexts, previous study involving a renal biopsy in an SLE patient revealed that patients with active SLE, such as those with positive results for anti-ds-DNA antibody and/or hypocomplementemia, had severe renal disease requiring immunosuppressive therapy, even if they had no clinical signs of renal disease — a condition called silent lupus nephritis [26]. Since our patient's findings were not consistent with these observations, as the patient presented with renal and hematological involvement and did not have anti-ds-DNA antibody or hypocomplementemia, diagnostic approaches for SLE, including considering the indication of a renal biopsy, were challenge.

In conclusion, we reported a diagnostically difficult cSLE boy with pancytopenia as the initial manifesta-

tion whose renal biopsy revealed lupus nephritis, which eventually led to the diagnosis of cSLE without anti-ds-DNA antibody or hypocomplementemia. Our case suggested the importance of renal biopsy for similar symptomatic patients without anti-ds-DNA antibody and/or hypocomplementemia, resulting in adequate diagnosis and treatment for those patients. The findings in the present case may implicate a potential pathophysiology of cSLE that does not involve anti-ds-DNA antibody or hypocomplementemia, which may help us better understand the diagnosis and treatment of cSLE. Because there have been few studies of SLE patients without anti-ds-DNA antibody or hypocomplementemia, further research is required to elucidate the features of such patients, including more subjects and a longer follow-up period in the future.

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DISCLOSURE STATEMENT

The authors declare that no financial or other conflicts of interest exist in relation to the contents of this article.

AUTHOR CONTRIBUTION

The authors confirm contribution to the paper as follows: study conception and design: YK, KK; data collection: TO, TS, KA, HT, DT, KH, HM; interpretation of results: YK, MK; draft manuscript preparation: YK, KK, MK. All authors reviewed the results and approved the final version of the manuscript.

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