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Unusual Association of Hemophagocytic Lymphohistiocytosis in Systemic Lupus Erythematosus: Cases Reported at Tertiary Care Center

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Case series
Patients: Female, 10 • Female, 15
Final Diagnosis: Secondary hemophagocytic lymphohistiocytosis
Symptoms: Arthralgia • CNS manifestations • fever • pancytopenia • rash
Medication: —
Clinical Procedure: —
Specialty: Hematology

Objective: Rare co-existence of disease or pathology

Background: Hemophagocytic lymphohistiocytosis (HLH) in the background of systemic lupus erythematosus (SLE) is rare. Inability to discriminate between these two entities may be fatal for the patient. Here we report two cases of SLE with secondary HLH, one of which manifested HLH as the initial presentation, and the significance of HLH's timely diagnosis.

Case Report: We describe two cases of SLE secondarily affected by HLH, which were diagnosed by various laboratory parameters and detection of profoundly reduced NK cell activity by using flow cytometry. Both our cases on investigation showed hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia, and marked reduction or complete absence of NK cell activity.

Conclusions: Association of secondary HLH with SLE is rare, and when it occurs, differentiating it from lupus flare requires a high degree of suspicion and awareness of this association. Both have overlapping clinical features, but HLH is characterized by hyperferritinemia, hypofibrinogenemia, hypertriglyceridemia, and a decrease in erythrocyte sedimentation rate (ESR) and NK cell activity unlike SLE. Therefore, early diagnosis of HLH in the background of SLE facilitates timely selection of an appropriate treatment modality to prevent fatal complications.

MeSH Keywords: Killer Cells, Natural • Lupus Vasculitis, Central Nervous System • Lymphohistiocytosis, Hemophagocytic

Full-text PDF: http://www.amjcaserep.com/abstract/index/idArt/899433

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Background

Hemophagocytic lymphohistiocytosis (HLH) is a disorder of immune dysregulation leading to systemic hyperinflammation due to uncontrolled proliferation of activated CD8 T cells, lymphocytes, and macrophages that secrete large amounts of inflammatory cytokines [1]. It is a rare, life-threatening condition usually affecting children and young adults [2]. The hyperactivation of the immune system causes varied clinical and laboratory abnormalities like fever, hepatosplenomegaly, pancytopenia, hyperferritinemia, hypertriglyceridemia, reduced or absent NK cell activity, and evidence of hemophagocytosis in bone marrow [3]. This disorder can be primary due to genetic defects leading to a decrease in the cytotoxic activity of NK cells or may be secondary to conditions like infections, malignancy, drugs, and autoimmune diseases [4]. Secondary HLH in patients with systemic lupus erythematous (SLE) is uncommon, with an estimated prevalence of 0.9–4.6% [5,6]. Hereby, we report two cases of HLH associated with SLE who presented to our hospital within a short span of two weeks. One was a follow-up case of SLE: a 10-year-old girl who presented with fever, cytopenias, and neurological involvement. The other was a case of undiagnosed SLE: a 15-year-old girl who presented with signs and symptoms of HLH.

Case Report

Case 1

A 10-year-old girl whose SLE had been followed up for 3 years presented to the pediatric outpatient department of our hospital and was admitted with complaints of fever of 6 weeks' duration. Fever was intermittent, moderate to high grade, and not associated with chills and rigors. She also gave a history of appearance of an erythematous rash and eruption involving both cheeks for the past two weeks. The rash gradually increased to involve the trunk and back. Following this, she developed tremors involving both upper and lower limbs of 3 days' duration. These were associated with dysarthria and inability to feed herself due to tremulousness, followed by an episode of generalized tonic clonic seizure (GTCS), which lasted for 5-7 minutes. During the postictal state she developed altered sensorium and inability to recognize her parents. There was no history of impaired vision, facial asymmetry, difficulty in swallowing or chewing, or nasal regurgitation to suggest cranial nerve palsies. No history was suggestive of joint swelling or bleeding diathesis. The patient had been a known case of SLE for 3 years, diagnosed on the basis of fever, rash, polyarthritis, positive antinuclear antibody (ANA), and positive anti-double stranded DNA (dsDNA) antibody and had been on immunomodulatory therapy (steroids and mycophenolate mofetil) since then. On admission the vital parameters were as follows: body temperature 102°F (38.9°C), pulse rate 120/min, respiratory rate 24/min, and blood pressure 104/74 mm Hg. Pallor was present; however, there was no lymphadenopathy, pedal edema, or icterus. Physical examination revealed erythematous maculopapular rash over the malar area of the face and trunk. No oral ulcers or joint swelling were noted. Systemic examination done for the chest and cardiovascular system (CVS) was within normal limits.

Central nervous system (CNS) examination revealed hypotonia and power of grade III/IV in all four limbs. No papilloedema was noted. Abdominal examination revealed mild hepatosplenomegaly. Laboratory investigations done at admission indicated hemoglobin 8 g/dL, total leucocyte count (TLC) 1000/mm^3, absolute neutrophil count 330/mm^3, and platelet count of 80,000/mm^3. Her erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were elevated to 70 mm/hr (normal range 1–20 mm/hr) and 15 mg/L (normal range <06 mg/L), respectively. The serum ferritin level was markedly increased to 45,395 ng/mL (normal range 15–150 ng/mL), and serum triglycerides were raised to 350 mg/dL. Her serum complement levels (C3, C4) were low, and a direct Coombs test was positive. Serological tests performed included VDRL, Widal, HIV, anti-HBsAg, anti-HCV, dengue, brucella, EBV, and leptospira, which were all negative. Her ANA titer was 1:320 with specked pattern. Repeated blood, urine, and stool cultures showed no growth. Her serum lactate dehydrogenase (LDH) was raised to 740 U/L (normal range 200–400 IU/L). Liver function tests were mildly deranged with aspartate transaminase (AST) being 640 U/L and alanine transaminase (ALT) at 550 U/L. The 24-hr urinary protein was 190 mg/24 hr, while the other renal parameters were normal. An echocardiogram (2D-ECHO) showed mild pericardial effusion with left ventricular ejection fraction (LVEF) of 55%. Computed tomography (CT) scan of the head showed generalized cerebral atrophy with no areas of infarction or meningeal enhancement. X-ray of the chest (PA view) was unremarkable. Importantly, NK cell cytotoxic activity assay with intracellular staining for perforin was performed by flow cytometry (FACS Calibur, Becton Dickinson, San Jose California, USA). We observed complete absence of intracellular perforin in NK cells ($CD3^-/CD56^+$) (Figure 1B) as compared to a healthy control (Figure 1A). The histology of bone marrow aspirate showed diffuse proliferation of macrophages with evidence of hemophagocytosis amidst the normal hematopoietic elements (Figure 2). Her classical clinical and laboratory features were consistent with a diagnosis of SLE with secondary HLH (Table 1). She was dilantinized, and broad-spectrum injectable antibiotics and antifungals were started in view of severe neutropenia. She was treated with pulsed methylprednisolone at a dose of 15 mg/kg for 3 days, thereafter tapered to tab prednisolone 1 mg/kg for 12 weeks. Tab cyclosporine was given in a dose of 3-5 mg/kg to maintain cyclosporine zero levels at 150-200 ng/mL until the patient attained remission after

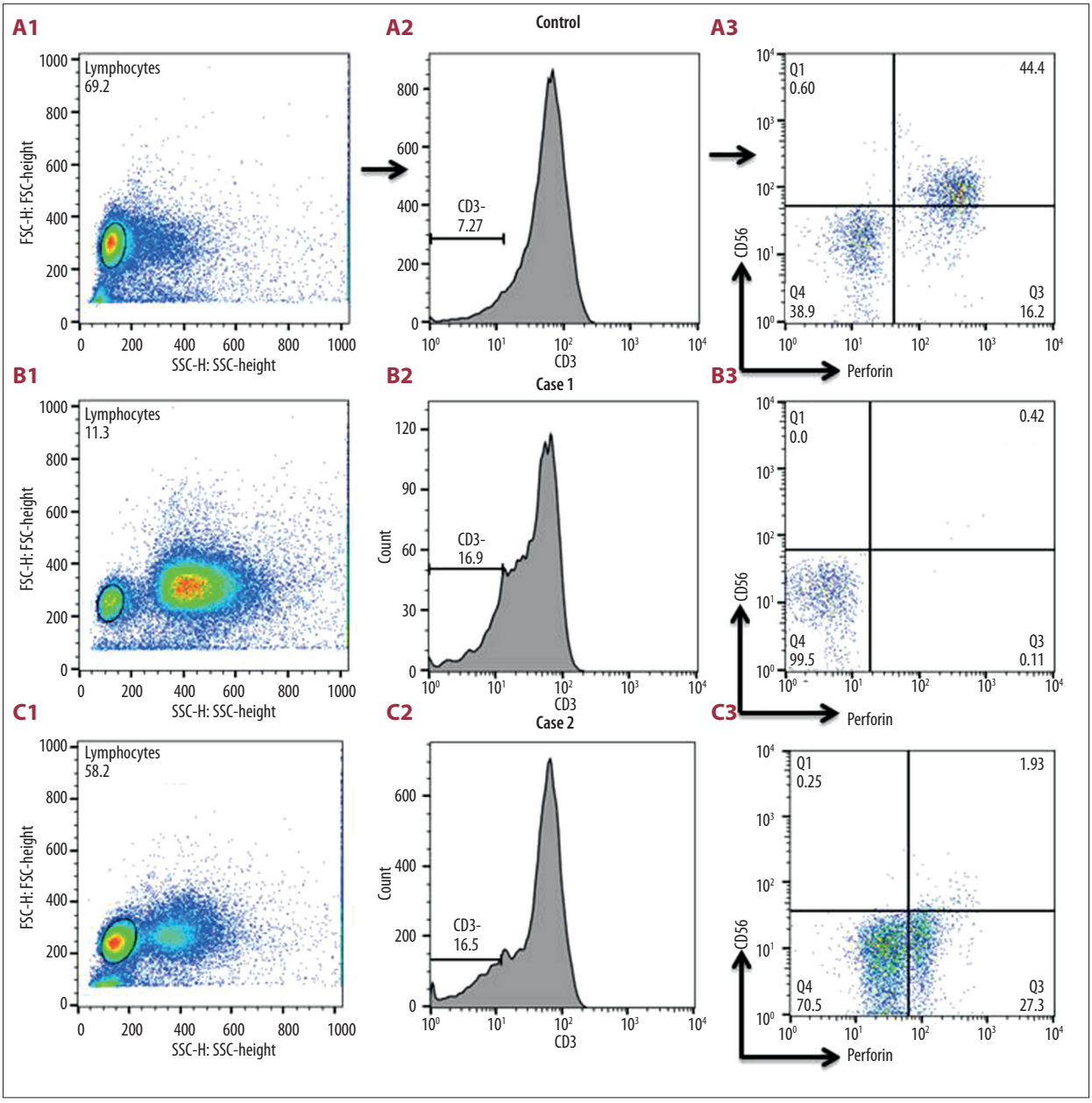

Figure 1. Immunophenotyping of NK cells and their cytotoxic activity from peripheral blood of patients (cases **B** and **C**) and the control (**A**) by using flow cytometry. Briefly, peripheral blood mononuclear cells were stained *ex vivo* with fluorescently labeled anti-human antibodies for cell surface (CD3 and CD56) and intracellular (perforin) markers, including anti-CD3 PeCy5, anti-CD56 PE, and anti-perforin FITC, by standard protocol. Cells were washed and acquired using BD FACS Calibur, and data were analyzed by FlowJo software. Lymphocytes were gated by forward (FSC) versus side (SSC) scatter (**A1, B1, C1**), and CD3-negative lymphocytes (**A2, B2, C2**) were further gated on CD56+ perforin+ (**A3, B3, C3**) NK cells highlighted in the top right quadrant of the dot plot.

4 weeks of treatment. The patient started improving clinically, her fever subsided, neurological signs and symptoms improved, and she became oriented in time, place, and person after 3 days. Gradually her laboratory parameters improved, with TLC recovering to 3700/mm^3 over a span of 2 weeks. Her serum ferritin decreased to 750 ng/mL in 3 weeks. Currently, the patient has been in remission for the last 6 months and is regularly followed up at our outpatient pediatric department.

Case 2

A 15-year-old girl with no comorbidities presented with fever of one month's duration. Fever was moderate to low grade,

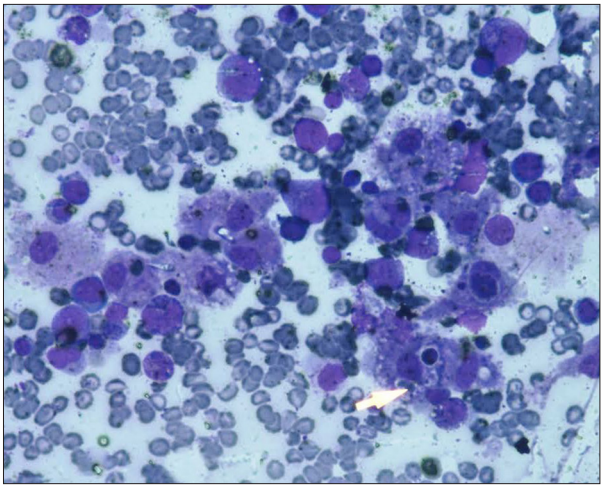

Figure 2. Hematoxylin and eosin (100×) section of bone marrow aspirate shows florid hemophagocytosis (indicated by arrow). The activated histiocytes show marked phagocytosis of all hematopoietic elements.

intermittent, and not associated with chills. She was being treated empirically with oral antibiotics by the local physician but had no relief. She developed oral ulcers and malar rash, and was referred to the medicine department of our center for further management. The patient also gave a history of alopecia, pedal edema, and photophobia. She also complained of polyarthralgia and myalgia. She had asymmetrical polyarthritis involving larger joints more than smaller joints, with axial sparing and no early morning stiffness. There was no history of any drug intake. On admission the vital parameters were as follows: body temperature 100°F (37.8°C), pulse rate 90/minute, respiratory rate 22/min and blood pressure 110/72 mm Hg. General physical examination revealed presence of pallor, bilateral enlarged cervical lymph nodes, and facial puffiness. Systemic examination of chest, CVS, and CNS was unremarkable. Abdominal examination revealed moderate hepatosplenomegaly. Laboratory investigations showed Hb 9 g/dL, TLC 2600/mm^3, platelets reduced to 100,000/mm^3, and ESR raised to 113 mm/hr. Biochemical parameters were also deranged, with raised liver function enzymes (AST 544 IU/L and AGT 266 IU/L), LDH 1683 IU/L (normal range 200–400 IU/L), and creatine phosphokinase 1644 U/L (normal range 5–130 U/L). The 24-hour urinary protein was 210 mg/day, but her other renal parameters like serum urea and creatinine were normal. Immunological screening was positive for ANA (1:160) with a homogenous pattern, anti-dsDNA, and direct Coombs test. Serum C3 and C4 complement factors were also low. Screening tests done included Widal, HIV, Anti-HBsAg, Anti-HCV, dengue, brucella, EBV, and leptospira to detect an infectious cause and were all found to be negative. Her triglycerides and ferritin were markedly elevated to 418 mg/dL and 8440 ng/mL, respectively. 2D-ECHO showed mild pericardial effusion with LVEF of 60%. Bone marrow aspirate was cellular reactive marrow with no evidence of hemophagocytosis. However, her NK cell intracellular perforin expression was markedly decreased (Figure 1C). The diagnosis of SLE was made with clinical and laboratory findings of malar rash, arthritis, photophobia, pancytopenia, positive ANA test, positive anti- dsDNA test and low complement levels (C3, C4). The patient fulfilled the American College of Rheumatology (ACR) and the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE. The patient also fulfilled six of the eight diagnostic criteria for HLH, which included fever, pancytopenia, hepatosplenomegaly, hypertriglyceridemia, hyperferritinemia, and reduced NK cell activity (Table 1). Hence our patient was diagnosed as a case of SLE with secondary HLH and was treated similar to the first case with pulsed methylprednisolone and oral cyclosporine. The patient started feeling better 2 days after the start of therapy. Cytopenias, i.e., TLC and platelets, normalized by day 3 and serum ferritin came down from 8440 ng/mL to 153 ng/mL in about 2 weeks. She is presently in remission and on regular follow-up at our outpatient pediatric department.

Discussion

Here, we discuss two pediatric cases of SLE with secondary HLH who presented with varied clinical features at our tertiary care center. The incidence of HLH with SLE is rare, about 0.9–4.6% [6], especially with HLH as the primary manifestation. HLH is a rare but potentially life-threatening disorder with dysregulation of the immune system. It is known to occur secondary to a large number of autoimmune conditions including systemic juvenile idiopathic arthritis (JIA), mixed connective tissue disorder, systemic sclerosis, Stills disease, etc. HLH developing in a patient with active SLE without evidence of underlying causes like infection or malignancies is known as acute lupus hemophagocytic syndrome. It is characterized by activation of lymphocytes and macrophages secreting high levels of cytokines, giving rise to its typical clinical and laboratory findings. Fever is caused by interleukins and tumor necrosis factor alpha (TNF-α). Ferritin is secreted by activated macrophages, which also produce high levels of plasminogen activator leading to hyperfibrinolysis. Cytokines like TNF-α and interferon gamma (IFN-γ) suppress lipoprotein lipase, causing triglyceridemia [7].

Both the cases diagnosed here had markedly decreased frequency of NK cells ($CD3^-/CD56^+$) expressing perforin within the intracellular compartment. Impaired NK cell cytotoxicity is a diagnostic hallmark of HLH to distinguish it from an SLE flare. Therefore, early diagnosis of HLH with SLE background facilitates timely selection of an appropriate treatment modality to prevent fatal complications. In immunocompetent individuals, NK cells and cytotoxic T lymphocytes (CTLs) both kill infected cells and antigen-presenting cells (APCs), resulting in clearance of pathogens (antigens) and termination of the

Table 1. Comparison of clinical and laboratory diagnostic parameters of the cases.

	Case 1	Case 2
Age	10 years	15 years
Sex	Female	Female
Fever	Febrile	Febrile
Hematological parameters		
Hb (g/dL)	8	9
TLC (mm^3)	1000	2600
Platelets (mm^3)	80,000	100,000
Hepatosplenomegaly	Present	Present
Hypertriglyceridemia (mg/dL)	350	418
Hyperferritinemia (ng/mL)	45,395	8440
Bone marrow hemophagocytosis	Present	Absent
Autoantibodies		
Anti-nuclear antibodies	1:320	1:160
Anti-dsDNA antibodies	Positive	Positive
Direct Coombs test	Positive	Positive
NK cell activity (flow cytometry-based intracellular perforin)	Absent	Markedly reduced
SLE organ involvement	Cutaneous, CNS, hematological	Cutaneous, hematological

immune response thereafter. Compromised NK cell function, including decreased or absent intracellular perforin, is believed to disrupt the immune regulation, resulting in excessive proliferation and activation of APCs. This plausibly leads to a cytokine storm due to excessive stimulation of lymphocytes including T cells, causing widespread organ damage including liver, spleen, bone marrow, brain, etc. [8].

Major clinical features of HLH are fever, cytopenias, and acute organ failure. Our first patient presented with fever followed by GTCS, altered sensorium, and inability to recognize people. CNS symptoms are seen in up to 75% of pediatric cases. These symptoms include seizures, meningitis, encephalopathy, ataxia, hemiplegia, cranial nerve palsies, mental status changes, or simply irritability. There have been reports of isolated CNS symptoms without accompanying systemic findings, termed cerebral HLH [9].

HLH in pediatric patients occurs mostly secondary to rheumatic diseases like JIA, when it is known as macrophage activation syndrome [10]. However in recent years an increase in cases associated with SLE has been reported. HLH in SLE usually presents in early stages of the disease or with disease onset, as was noted in our second case. In JIA, it occurs at advanced stages of the disease [11,12]. Both our patients had a prolonged duration of fever not associated with an infectious process and did not respond to broad-spectrum antibiotics. Pancytopenia occurring in the background of SLE with high ferritin levels is rare and was seen in both our cases, which is highly suggestive of HLH. The diagnosis of HLH secondary to SLE is complicated as both have overlapping features, but HLH has certain distinct laboratory findings distinguishing it from SLE. Hyperferritinemia is considered as most important parameter that differentiates HLH from SLE and has a high sensitivity and specificity of 100% [13].

A study by Wang et al. [14] explored the clinical significance of NK cell activity and soluble CD25 in serum from patients with secondary HLH and also concluded that NK cell activity and sCD25 levels may be valuable in early diagnosis of secondary HLH. Moreover, bone marrow trephine biopsy helps to confirm hemophagocytosis; however, it is important to note that absence of hemophagocytosis can occur in 20% of initial bone marrow specimens, as was noted in our second case [15].

Early diagnosis of HLH is important as timely initiation of immunomodulatory therapy can indeed be life saving. The goal of therapy for patients with HLH is to suppress the hyperinflammation by destroying the immune cells. The Histiocyte Society proposed an intensive eight-week induction regime with dexamethasone, etoposide and intrathecal methotrexate [16]. Cyclosporine has been found to be effective in severe or steroid-resistant cases of HLH [17]. Biological agents, i.e. anti-TNF-α, anti IL-7, rituximab, and anti-CD3 are being considered as alternatives for treatment [18] in refractory cases of HLH.

The clinicians need to be confident in the disease diagnosis before initiating any aggressive regime. Our patients responded to high-dose pulsed methylprednisolone and oral cyclosporine, recovered and are presently on regular follow-up at the outpatient department, All India Institute of Medical Sciences.

Conclusions

HLH complicating SLE can have similar or overlapping clinical manifestations, thus delaying the disease diagnosis and timely intervention, which can be life-threatening for the patient.

One of the easy-to-perform diagnostic flow cytometry-based assay is the determination of NK cell activity. This test can be carried out in a well-equipped laboratory and provides an early diagnostic clue to HLH and its pathogenesis. A high index of suspicion on the part of the clinician supported by laboratory investigation for NK cell activity confirms HLH and allows the selection of an appropriate treatment regime, which can go a long way in saving the lives of these patients.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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