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THERAPEUTICAL PRACTICE

MACROPHAGE ACTIVATION SYNDROME IN TWO GIRLS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Abstract. Background: Most frequently seen in Systemic Arthritis – Juvenile Idiopathic Arthritis, macrophage activation syndrome (MAS) may also be a life-threatening complication in juvenile systemic lupus erythematosus (SLE). The diagnosis of MAS may be particularly challenging because it may mimic the clinical and laboratory features of the underlying disease. Objective: To describe the clinical and laboratory features of MAS as an early complication of juvenile systemic lupus erythematosus. Methods: We report two cases of females with MAS in Juvenile SLE occurring acutely in the first 6 months after the onset, at the time of first presentation in our hospital. The clinical features and laboratory data were analyzed. The main laboratory findings of MAS were present: pancytopenia, abnormal serum hepatic enzyme levels, coagulopathy, neurologic symptoms, hyperferritinemia, hypertriglyceridemia, decreased erythrocyte sedimentation rate, hyponatremia, hypoalbuminemia and macrophage hemophagocytosis in the bone marrow aspirate sample. The treatment included intravenous methylprednisolon, immunoglobulins, cyclophosphamide pulse (one case), transfusions (PT, FFP, PRC) and supportive therapy. Conclusion: The diagnosis of MAS can be difficult because some of its clinical features overlap those of lupus itself. The occurrence of unexplained fever and pancytopenia associated with increased hepatic enzyme levels, coagulopathy, hyperferritinemia should promptly raise the suspicion of macrophage activation syndrome.
Keywords: MAS, SLE, children

Background

Macrophage activation syndrome (MAS) is a life-threatening complication of rheumatic disease [1]. The diagnosis of MAS may be particularly challenging in patients with systemic lupus erythematosus (SLE) because it may mimic the clinical and laboratory features of the underlying disease [2-5]. Macrophage activation syndrome is characterized by pancytopenia, liver insufficiency, coagulopathy, and neurologic symptoms and is thought to be caused by the activation and uncontrolled proliferation of T lymphocytes and well-differentiated macrophages, leading to widespread hemophagocytosis and cytokine overproduction [1,2,6].

Objectives

To describe the clinical and laboratory features of MAS as an early complication of juvenile systemic lupus erythematosus.

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Methods

We report two cases of 15- and 10-year-old females with MAS in juvenile SLE occurring acutely in the first 6 months after the onset at the time of first presentation in our hospital. The clinical features and laboratory data of SLE were analyzed. The main laboratory findings of macrophage activation syndrome were identified: pancytopenia, abnormal serum hepatic enzyme levels, coagulopathy, neurologic symptoms, hyperferritinemia, hypertriglyceridemia, decreased erythrocyte sedimentation rate, hyponatremia, hypoalbuminemia and macrophage hemophagocytosis in the bone marrow aspirate sample.

Case 1.

A 15 year old girl with SLE onset three months ago was admitted on 03/17/2010 with fever, malar rash, oral ulcerations, laterocervical enlargement of lymph nodes, arthritis, myalgia, lumbar pain, pancytopenia, lethargy, irritability, disorientation, headache, epistaxis, and secondary amenorrhea. Before the admission in our hospital she was treated many times for tonsilitis, sinusitis, „minor post-streptococcal disease" (ASO 701 iu/mL) at the local hospital. Prolonged fever and the signs above mentioned were considered for a possible collagenosis and the patient was sent to our department of pediatrics.

The diagnosis of SLE met 6 of the American College of Rheumatology 1997 criteria: malar rash, oral mucocutaneous ulcerations, nonerosive arthritis (knees, wrists, bilateral PIP 2-5 joints), encephalopathy (psychosis), cytopenia (pancytopenia), positive immunoserology (**positive antibody anti-nucleosome, anti-Sm, anti-Sm/RNP**, but **negative anti-dsDNA and ANA** Screen).

Vital signs at admission were normal: blood pressure of 104/70 mmHg, heart rate of 70/min, respiration rate of 16/min, and body temperature of 36,5°C. On physical examination, she had fever, earthly, dry, desquamate teguments, malar rash, laterocervical enlargement of lymph nodes, anemic conjunctivae, oral ulcerations, dehydrated tongue, and mild hepatosplenomegaly. She had also multiple joint swelling and tenderness. Her mother descriebes in the last week episodes of lethargy, irritability, disorientation, headache, and epistaxis. Secondary amenorrhea in the last three months was noted.

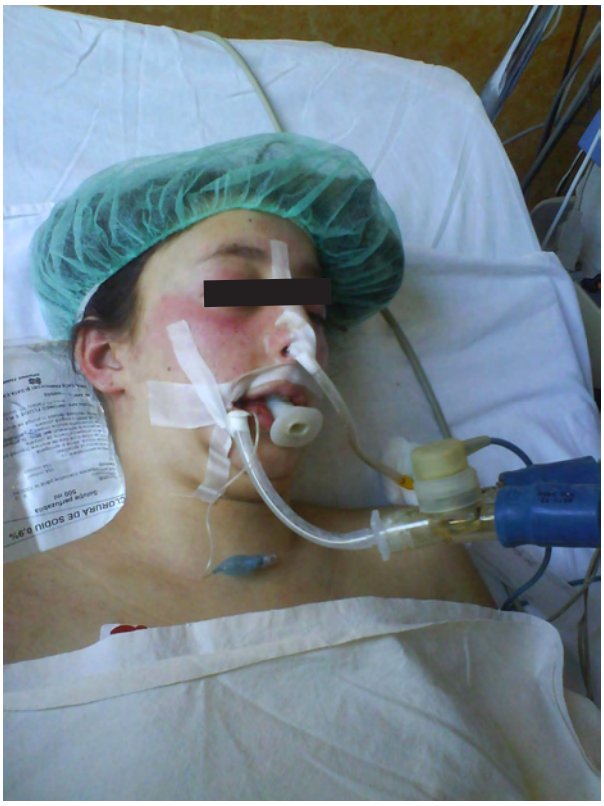

Figure 1. Girl with SLE

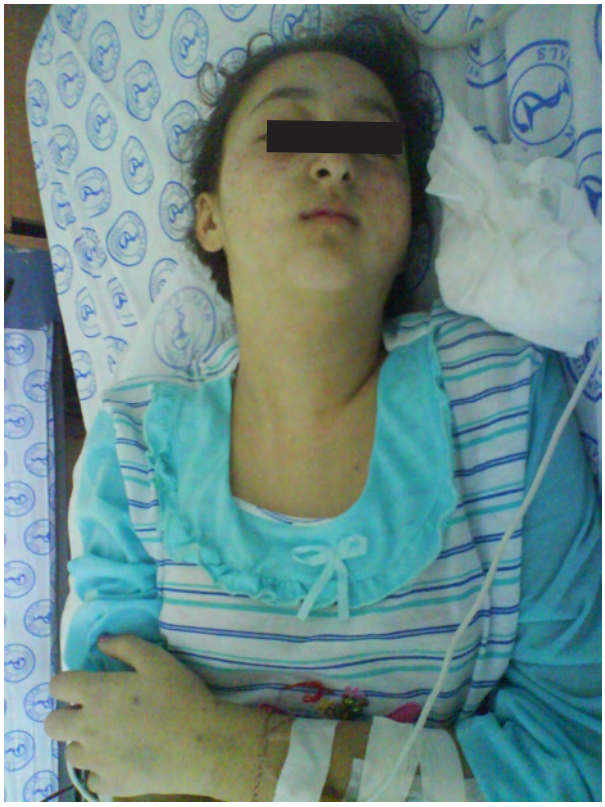

Figure 2. Rash morbilliforme

Laboratory data

Table I summarizes the laboratory findings for both cases.

Findings	Case 1(3/18/2010)		Case 2(5/18/2010)	
Pancytopenia	+	WBC2.71*10^3/μL PLT 83*10^3/μL RBC1.81*10^6 /μL Hb 4.8g/dL	+	WBC1.13*10^3/μL PLT 80*10^3/μL RBC3.05*10^6 /μL Hb 6.8g/dL
Increased liver enzymes (e.g. AST, ALT)	+	AST 1,107 U/L ALT 347 U/L	+	AST 172 IU/L ALT 196 IU/L,
Increased lactate dehydrogenase(LDH)	+	1069 IU/L	+	2381 IU/L,
Hypertriglyceridemia	+	381mg/dL	+	748 mg/dL
Hypofibrinogenemia	+	175 mg/dL	+	100 mg/dL
Hyperferritinemia	+	4233 μg/L	+	12,983 μg/L
Decreased albumin	+	2.8 g/dL	+	2.4 g/dL
Hyponatremia	+	133mmol/L	+	132 mmol/L
Increased D-dimers	+	1,274 ng/mL	+	5,785 ng/mL
Prolongation of clotting times	-	Normal values(?)	-	Normal values(?)
Bone marrow haemophagocytosis	+		+	See picture 4, 5, 6
Antinuclear antibodies	-	Negative	+/-	Gray zone
Anti-DNA antibodies	-	Negative	+/-	Gray zone
Antinucleosome antibodies	+	36 U/mL	+	37.8 U/mL
Decreased C3	+	37mg/dL	-	141mg/dL
Falling erythrocyte sedimentation rate	+	18 mm/h	+	12 mm/h
High C-reactive protein	+	1.53 mg/dL	+	9.8 mg/dL

Table I. Normal values include: AST <84 IU/L; ALT <65 IU/L; LDH 90-190IU/L; Triglycerids 35-160mg/dL; Ferritin 7-140μg/L; D-dimers 0-230 ng/mL; Antinucleosome antibodies: negative < 20 U/mL; C3 90-180mg/dL

Other laboratory results included: GGT-347 U/L(5-85), ALKP-189 U/L(70-230), CK-929 U/L(24-170), Amylase-492 U/L(30-100), BUN-78mg/dL, Cr -0.6mg/dL, and uric acid-7.7mg/dL. Coagulation tests and disseminated intravascular coagulation profiles showed prothrombin time (PT) of 102% (80-115), activated partial thromboplastin time (APTT) 31.8 sec (24-38 sec), fibrin degradation product (FDP) positive, antithrombin III 120%, Protein C 115% (70-130), Free Protein S 59.3 (54-123) and INR 0.99 (0.90-1.15). Lupus anticoagulant, anticardiolipin and anti Beta 2-Glycoprotein were negative.

Serological tests for viral infections, such as viral hepatitis, Epstein-Barr virus, cytomegalovirus and herpes simplex virus, revealed no sign of recent infections. Bacterial cultures of blood and urine and also PCTQ (procalcitonine) were all negative. Lumbar puncture shows a normal CSF. Bone marrow aspiration and biopsy showed marked increased histiocytes with active hemophagocytosis (Fig. 1). We diagnosed her as having macrophage activation syndrome associated with SLE. Intravenous pulsed methylprednisolone (1 g/day for three days) was administered. However, his symptoms and clinical signs did not improve, but on the contrary a fulminant gastrointestinal bleeding with haemor-

rhagic shock and lost of consciounsness occur. Eye examination demonstrated papilar edema, multiple peripapilar and perivascular hemorrhagia suggesting retinal vasculitis. MRI showed. The treatment consisted of transfusions (platelets, FFP, and PRC) intravenous methylprednisolon, immunoglobulins, cyclophosphamide pulse, and supportive therapy (intubation and mechanical ventilation for three days).

After 9 days, pancytopenia recovered with white blood cells count 5,000/mmc, hemoglobin 9.6 g/dL, hematocrit 28.6% and platelet count 157,000/ mmc. Deteriorations of liver and renal function were also improved with AST 100 IU/L, ALT 108 IU/L, total bilirubin 1.89 mg/dL, BUN 31 mg/dL and Cr 0.5 mg/dL, and serum ferritin decreased to 1,193 μg/L.

During the next three months, clinical and biological recovery was near complete under combined treatment with prednison and intravenous pulsetherapy with cyclophosphamide. Although the patient was asymptomatic, at the end of the third month of treatment, surprising high ESR (77mm/h) and CRP (25.5mg/dL) were find, and chest radiograph shows a miliary tuberculosis.

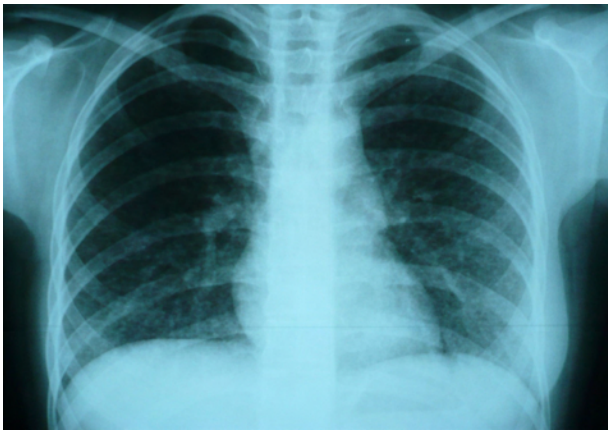

Figure 3. Chest Radiograph

Case 2.

A 10 year old girl with SLE onset two months ago, was admitted on 06/02/2010 with fever, rash, arthritis, myalgia, lethargy, irritability, disorientation. The onset was two months ago, short time after a mild forme of varicella, with arthritis (right hip, ankles, PIP), high fever (<39.5°C), progressive generalized rash, itching, swollen ankles, myalgia, cough and purulent rhinorrhea. After repeated apparent successful treatments with antibiotics for lower respiratory infections (ceftazidinum and gentamicinum, cefoperazonum with sulbactam), or for pansinusitis (ceftriaxonum), high fever and chills recurred between 15 and 17 of May 2010. On 05/18/2010 an acute worsening occurs, with abdominal pain and vomiting, malaise, irritability, bizarre behaviour, disorientation, recurrent generalized seizures and coma (Glasgow Score 7-8). The patient was admitted in the National Institute for Infectious Disease „M. Balş" with the suspicion of acute encephalitis (fever, seizures, coma, nystagmus, anisocoria, right cranial nerve VI palsy), but in the next days macroscopic hematuria, proteinuria, morbilliforme rash, and, after the recovery of consciousness, joint pain and myalgia occured.

The diagnosis of SLE met 6 of the American College of Rheumatology 1997 criteria: rash (morbilliforme), nonerosive arthritis (hip, ankles, PIP), nephritis, encephalopathy (psychosis, seizures, coma), pancytopenia and positive immunoserology (**positive antibody anti-nucleosome with 37.8 U/mL, but gray zone values for anti-Sm, anti-Sm/RNP**, **anti-dsDNA and ANA** Screen).

Vital signs at admission: blood pressure of 108/67 mmHg, heart rate of 108 bpm, respiration rate of 28/min, and body temperature of 39°C. On physical examination, she had fever, morbilliform rash, arthritis, myalgia, anemia, complex partial seizures (masticatory automatisms), bilateral pyramidal syndrome, right sixth nerve palsy, right vertical nystagmus, anisocoria, lethargy, irritability, disorientation.

Like in the first case, the patient had all components that showed the highest sensitivity and specificity for MAS (see Table I): increased serum ferritin, triglycerides, ALT, AST, gamma-glutamyl transferase; decreased fibrinogen, and platelet count; bone marrow aspirate showing macrophage hemophagocytosis (Fig. 4, 5, 6). Other investigations included: BUN 30.2mg/dL, Cr 0.8mg/dL, and uric acid 5mg/dL. Direct Coombs test was positive. Total bilirubin 0.5mg/dL. Coagulation tests and disseminated intravascular coagulation profiles showed prothrombin time (PT) of 105 % (80-115), fibrin degradation product (FDP) positive, Protein C 116% (70-130), Free-Protein S 30.2 (54-123) and INR 0.92 (0.90-1.15). Lupus anticoagulant-LAC-SCREEN(R) 1.35 (0.9-1.1 ratio); anti-cardiolipin and anti-Beta 2-Glycoprotein antibodies were negative.

Serological tests for viral infections, such as viral hepatitis, coxsackie, ECHO, Epstein-Barr virus, adenovirus, cytomegalovirus and herpes simplex virus, revealed no sign of recent infections. Bacte-

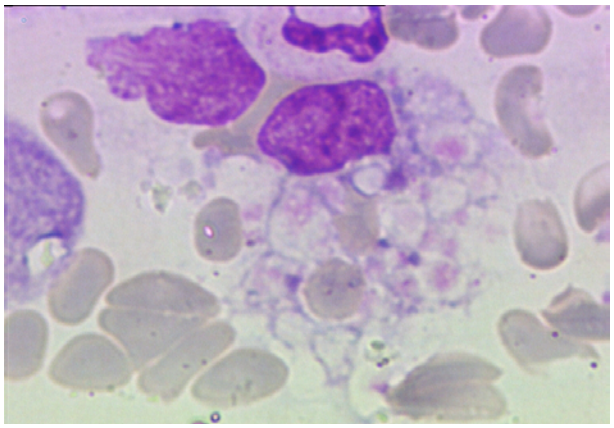

Figure 4. Macrophage with phagocytated platelets, erythrocytes and lipids (resulted from distruction of cell membrans). Giemsa 1000X

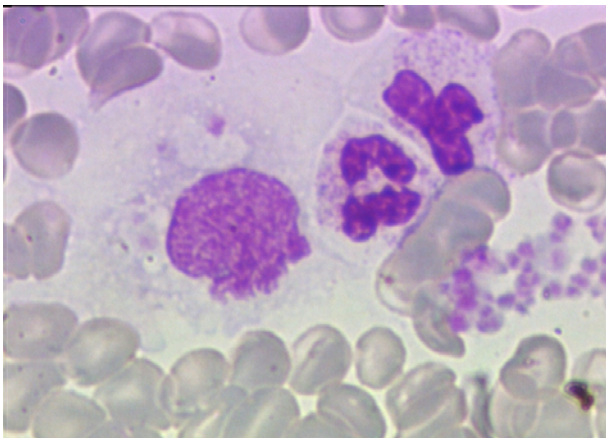

Figure 5. Macrophage with phagocytated platelets and lipids. Giemsa 1000X

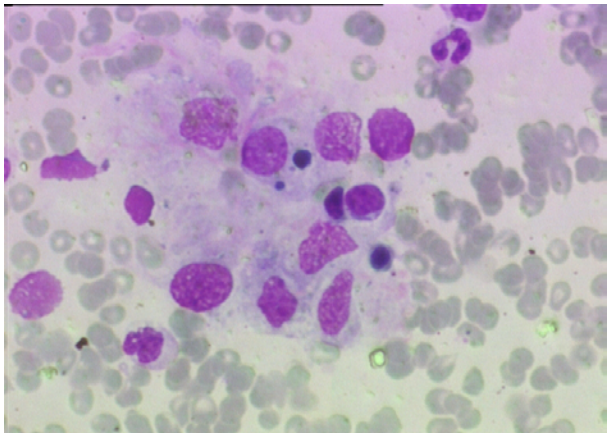

Figure 6. Macrophage with hemosiderine (iron granules) in cytoplasm. Giemsa 400X

rial cultures of blood and urine and also PCTQ (procalcitonine) were all negative. Lumbar puncture shows: Pandy Test positive (+), 30 elements/mmc, total proteins 100mg/dL, and normal glucose and chloride. Bone marrow aspiration showed increased macrophages with a mild active hemophagocytosis 19 days after the onset of MAS (Fig. 4, 5 and 6). Angio MRI: bilateral diffuse demyelinating lesions of supratentorial white matter with mainly involvement of bilateral white external capsule and bilateral posterior arm of the internal white capsule; thickening of cranio-facial sinusal mucosa with fluid collection in maxillary and sphenoidal sinuses.

Intravenous pulsed methylprednisolone (1 g/day for three days) and IVIG (20g/day for five days) were administered. Seizures were treated with phenytoinum, levetiracetanum and phenobarbitalum. She received also meropenemum, linezolidum to treat pansinusitis. The recovery was delayed because the patient suffers an episod of acute superficial thrombophlebitis of the right shank treated with ibuprofenum, nadroparinum (4 days), and teicoplaninum. So while platelet count was spontaneous recovered, anemia needed transfusions (PRC), and the persistent leucopenia with severe neutropenia (450/mmc) recovered after three doses of filgrastinum, a month after the onset. Deteriorations of liver function was also improved with AST 40 IU/L, ALT 100 IU/L, and serum ferritin decreased to 1127µg/L.

Three weeks after admission the patient is established and leaves the hospital with recommendation to continue steroidal treatment (prednison) combined with azathioprine and also antiepileptic treatment with levetiracetanum.

Discussion

It has been recently recognized that MAS belongs to secondary hemophagocytic syndromes [1, 2, 6, 7] histiocytic disorders associated with an underlying systemic disease: JIA (most common), SLE, vasculitis (Kawasaki disease), adult-onset Still's disease, and Behcet's disease.

The exact pathogenic mechanisms involved in the development of MAS are not known. It appears that there is an underlying abnormality in immunoregulation that contributes to the lack of control of an exaggerated immune response. Indeed, the clinical findings during the acute phase can largely be explained as a consequence of the prolonged production of cytokines and chemokines originating from activated macrophages and T cells.

An immune response in MAS can be triggered by a viral infection. NK cells fail to limit the extent of viral replication at early stages of infection leading to increased viral load and more massive expansion of cytotoxic CD8+ cells [2, 6, 9, 10]. At later stages due to perforin deficiency and/or poor NK cell function, cytotoxic CD8+ cells are not eliminated even if infection is cleared (perforin is a cytotoxic protein that lymphocytes secrete to kill virus-infected cells and has a function that control the lymphocyte proliferation; therefore, the perforin deficiency may lead to persistent lymphocyte activation). They continue to survive and secrete proinflammatory cytokines [2, 6, 8, 9]. Prolonged stimulation of macrophages with cytokines leads to their excessive activation and proliferation associated with hemophagocytic activity and production of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6. The serum macrophage colony-stimulating factor (M-CSF) and IL-18 levels were substantially elevated in all the patients with MAS, but in those with SLE, the M-CSF level was higher than the IL-18 level [10]. Hemophagocytosis of blood elements in the bone marrow leads to peripheral cytopenias. In fact, hemophagocytosis, the pathognomonic feature of the syndrome, is a hallmark of cytokine-driven excess activation of macrophages [6, 9]. This storm likely has a role in later sequelae including hepatic triaditis*, and central nervous system demyelination, like in our the second case [6]. It has been explained that the over-activated T lymphocytes and macrophages are found in various organ [2, 6].

The diagnosis of MAS may be particularly challenging in patients with SLE because it may mimic the clinical and laboratory features of the underlying disease [3, 4, 5]. The sensitivity and specificity of the main components of the clinical and laboratory features of MAS were recently determined [2, 9]. The variables that showed the highest sensitivity and specificity for MAS were the following: serum ferritin ≥10,000 ng/mL, triglycerides ≥160 mg/dL, SGOT ≥40 IU/mL, fibrinogen ≤250 mg/dL, SGPT ≥40 IU/mL, gamma-glutamyl transferase ≥40 IU/mL, platelet count ≤150,000/L, bone marrow aspirate showing macrophage hemophagocytosis, hepatomegaly, and splenomegaly [2, 10]. In our case, except hepatosplenomegaly, the patient had all components that showed the highest sensitivity and specificity for MAS, mentioned above.

A constellation of inflamed organs that are adjacent each other, namely the small intestine, liver, and pancreas

Hyperferritinemia is an important laboratory hallmark of macrophage activation syndrome that has received increasing attention; elevated ferritin levels (often >10,000 ng/mL) have been reported in the acute phase of macrophage activation syndrome. Furthermore, a good correlation between ferritin levels and response to therapy has been observed; a decrease in ferritin levels is associated with a favorable course of macrophage activation syndrome. Recent studies have shown that low levels of glycosylated ferritin, in the presence of high level of total serum ferritin, may be another helpful marker for diagnosis [2, 12].

Why our patients did not have positive or had insignificant positive (gray zone) antibodies antinuclear, and anti-dsDNA, but positive antinucleosome antibody, remains a question. We think that this association may be random.

Because MAS is a serious condition that can follow a rapidly fatal course, prompt recognition of its clinical and laboratory features and immediate therapeutic intervention are critical. It has been suggested that the initial treatment of choice in MAS is high-dose corticosteroid, but sometimes MAS may appear to be corticosteroid resistant. The administration of high-dose intravenous immunoglobulins, cyclophosphamide, plasma exchange, and etoposide has provided conflicting results [2]. Increased production of tumor necrosis factor (TNF) in the acute phase of MAS has suggested the use of TNF-α inhibitors as potential therapeutic agents [13], although other investigators have observed the onset of MAS in patients with systemic juvenile idiopathic arthritis (SJIA) who were treated with etanercept [14] or with the recombinant interleukin (IL)-1 receptor–antagonist anakinra [15]. Although the association between MAS onset and treatment with etanercept or anakinra may be coincidental and not causal, the above-mentioned observations suggest that inhibition of TNF or IL-1 does not prevent MAS [2].

Recently, cyclosporine A (CyA) proved effective in treating severe or corticosteroid-resistant MAS [16]. Because of the distinctive efficacy of CyA, some authors have proposed using this drug as the first-line treatment for MAS occurring in childhood systemic inflammatory disorders [2], but therapeutic protocols are not yet suitable for use in children.

In the absence of CyA, initially we treated both cases with high dose methylprednisolonum (pulse

therapy) and intravenous immunoglobulins (IVIG) 20g/d for 5 days. In our first case, the abrupt worsening occuring in the third day of the treatment with high-dose corticosteroid (fulminant gastrointestinal bleeding with haemorrhagic shock and lost of consciounsness), imposed intravenous pulsetherapy with cyclophosphamide. The evolution in the next two months was satisfactory under prednison (oral) and monthly intravenous cyclophosphamide for SLE, but although the patient remained asymptomatic, at the end of the third month of treatment, surprising high ESR (77mm/h) and CRP (25.5mg/dL) were find, and chest radiograph shows a miliary tuberculosis. In the second case we assisted at a very slowly clinical and biological recovery after the treatment with high-dose corticosteroid, IVIG, and then with oral prednison (after three weeks serum ferritin decreased to 1127 μg/L, triglycerids to 332mg/dL, and a mild hemophagocytosis in the bone marrow is yet present). Cyclophosphamide couldn't be administred because a very severe neutropenia persisted for three weeks. After its recovery with filgrastinum, a combined treatment with prednison and azathioprinum for SLE was followed. Also the patient continues antiepileptic treatment with levetiracetanum.

The presence of MAS in these two cases with juvenile SLE occurring acutely in the first 6 months after the onset is an remarkable fact. To our knowledge, these are the first cases of MAS reported in patients with juvenile systemic lupus erythematosus in Romania.

Conclusions

Because MAS is a serious condition that can follow a rapidly fatal course, prompt recognition of its clinical and laboratory features and immediate therapeutic intervention are mandatory. The early diagnosis can be difficult, and differential diagnosis between MAS and a lupus flare or between MAS and infections is sometimes critical.

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