Macrophage activation syndrome in 13 children with systemic-onset juvenile idiopathic arthritis

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Background: Macrophage activation syndrome (MAS) is a severe, potentially life-threatening condition induced by chronic rheumatic diseases, especially systemic-onset juvenile idiopathic arthritis (SoJIA) in childhood. This study aimed to analyze the clinical and laboratory characteristics of systemic-onset juvenile idiopathic arthritis (SoJIA) with macrophage activation syndrome (MAS) in 13 patients.

Methods: Clinical and laboratory data of 13 SoJIA patients with MAS treated in our hospital from January 2003 to October 2007 were analyzed.

Results: In the 13 patients, 9 were boys and 4 girls aged from 5 months to 12 years. Clinical manifestations were of no typical characteristics including persistent fever, anemia, arthritis, hepatosplenomegaly, lymph-adenopathy, dysfunction of the liver, abnormal fat metabolism, and hemophagocytic cells in the bone marrow. Two patients experienced acute respiratory distress syndrome, two had mutiorgan failure, and three died. The perforin A91V (NCBI:SNP rs35947132) gene in 6 patients was normal. Glucocorticoid and immunoimpressive therapy were effective in all patients and plasmapheresis used in one severe patient was also effective.

Conclusions: MAS is a serious complication of JIA, especially systemic-onset juvenile idiopathic arthritis. It is essentially important to recognize and treat MAS earlier in order to lower the mortality.

Introduction

Macrophage activation syndrome (MAS) is a severe, potentially life-threatening condition induced by chronic rheumatic diseases, especially systemic-onset juvenile idiopathic arthritis (SoJIA) in childhood. It is characterized by the uncontrolled activation and proliferation of T cells and excessive activation of macrophages, resulting in persistent high fever, hepatosplenomegaly, lymphadenopathy, severe cytopenia, serious liver disease, intravascular coagulation, and neurological involvement. SoJIA constitutes about 10%-20% of all cases of JIA. However, more than two thirds of deaths in JIA patients are due to SoJIA. MAS, also a form of secondary hemophagocytic lymphohistiocytosis (HLH), is a major cause of morbidity and mortality in children with SoJIA.[1] Two recent research reports give a mortality of 8%-22%.[2,3] Hence, earlier diagnosis and treatment of MAS are important to decrease the mortality of children with SoJIA.

Methods

Patients

Clinical data were collected from 13 patients with SoJIA complicated by MAS at our hospital from January 2003 to October 2007. All the patients were identified as having SoJIA according to the classification criteria of the International League of Associations for Rheumatology (ILAR). Because there were no formal and universally accepted criteria for the diagnosis of MAS, we (as many clinicians do in practice) used the HLH criteria. The HLH criteria formulated in 1991 by members of the Histiocyte Society included clinical and laboratory criteria: fever (duration $\geq$7 days, with peaks $\geq$38.5ºC); splenomegaly ($\geq$3 cm below the costal arch); cytopenia (affecting $\geq$2 to 3 lineages in the peripheral blood and not caused by a hypocellular or dysplastic bone marrow): hemoglobin <90 g/L, platelets <100×10^9/L, neutrophils <1×10^9/L; and hypertriglyceridemia and/or hypofibrinogenemia (fasting triglycerides $\geq$2.0 mmol/L or $\geq$3SD the normal

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value for age and/or fibrinogen ≤1.5 g/L or ≤3SD). Histopathologically, there was hemophagocytosis in bone marrow or spleen or lymph nodes, and no evidence of malignancy.[4]

In the 13 patients, 9 were boys and 4 girls aged from 5 months to 12 years. The clinical data (symptoms, signs, laboratory features, treatment and outcome) of the patients were analyzed retrospectively. Because of the small number of patients, descriptive statistical analysis was made. The study was approved by the Institutional Ethics Committee of the hospital and informed consents were obtained from the parents or relatives of the patients.

DNA extraction
Polymorphisms of perforin A91V (NCBI:SNP rs35947132)

Table 1. Clinical data at diagnosis of the 13 patients

Clinical manifestations	Number of patients (%)
Fever >39ºC	13 (100)
Liver enlargement	12 (92.3)
Spleen enlargement	10 (76.9)
Lymphadenopathy	8 (61.5)
MODS	10 (76.9)
MOF	3 (23.1)
ARDS	2 (15.4)

MODS: multiorgan disfunction syndrome; MOF: multiorgan failure; ARDS: acute respiratory distress syndrome.

Table 2. Laboratory investigations

Biological data	Number of patients with abnormalities during MAS	Mean
Hemoglobin (g/L)	12	90.4
WBC ($\times 10^9$/L)	7	4.5
Platelets ($\times 10^9$/L)	7	51
Alanine aminotransferase (μ/L)	10	153.6
Triglyceridemia (mmol/L)	10	4.2
Fibrinogen (g/L)	7	1.47
ESR (mm/h)	9	32
Serum ferritin (ng/ml)	8	>1500
Lactate dehydrogenase (μ/L)	13	521
Hemophagocytosis (marrow)*	13	-

*: bone marrow aspirates in all patients during MAS. WBC: white blood cell; ESR: erythrocyte sendiment rate.

Table 3. Pathogens of 13 patients

Pathogens	Number of patients
Infections	3
EBV	1
CMV and HSV-I	1
CBV	1

EBV: epstein-barr virus; CMV: cytomegalovirus; HSV-1: herpes simplex virus 1; CBV: coxsackie B group virus.

were detected in 4 patients. Genomic DNA was extracted from 200 μl of whole blood with QIAGEN QiaAmp mini extraction kits (Qiagen, Krefeld, Germany) in accordance with the manufacturer's instructions.

Genotyping
Genotyping for the perforin A91V (NCBI:SNP rs35947132) polymorphisms was achieved following the procedures: polymerase chain reaction (PCR), PCR products purification and sequencing. Specifically, primers for the perforin A91V were as follows: forward, 5'CACCCTCTGTGAAAATGCCCTAC'3 and reverse, 5'TTCCAGTCGTTGCGGATGCTAC'3. PCR reactions were run at 95ºC for 5 minutes, followed by 35 cycles at 95ºC for 30 seconds, at 60ºC for 45 seconds, at 72ºC for 60 seconds, and a final incubation at 72ºC for 5 minutes. A 171-bp fragment of the perforin gene including the second intron was amplified. Each PCR was carried out in 25 μl of reaction mixture containing 25-50 ng genomic DNA and 12.5 μl of GoTaq® Green Master Mix (Promega, Madison, USA), 20 pmol/L of primers (Sangon, Shanghai, China) by the thermocycler (Bio-Rad, MyCycler, USA). PCR products were then purified and sequenced in an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Results
Clinical manifestations
Fever was observed in all the patients with a duration of 16 days to 3 months, and the common types of fever were continued fever, remittent fever and intermittent fever. Respiratory infections were found in 3 patients. Their common signs were hepatomegaly (12/13), splenomegaly (10/13), and enlargement of lymphonodes (8/13). Complications were common. Multiorgan disfunction syndrome (MODS) occurred in 13 patients, multiorgan failure (MOF) in 3, acute respiratory distress syndrome (ARDS) in 2, pneumohemorrhagia in 3, and dermo-mucosal bleeding in 2 (Table 1).

Laboratory tests
The mean level of hemoglobin was 90.4 g/L. Pancytosis occurred in 7 patients. All the 13 patients had a decreased level of hemoglobin. Abnormal liver function was tested commonly. Alanine aminotransferase >150 μ/L (normal: 12-40 μ/L) in 10 patients, and aspartate aminotransferase >100 μ/L (normal: 12-40 μ/L) in 12 patients. Hyperglycemia occurred in 10 patients and abnormal blood-fat was also commonly seen, i.e., decreased HDL-C in 8, and elevated LDL-C in 7. The level of serum ferritin

increased in 8 patients and that of FIB decreased in 7 (Table 2). Three patients showed positive etiological results: CBV-IgM(+) in one patient, CMV-IgM(+) and HSVI-IgM(+) in one, and EBV-IgM(+) in one (Table 3). The 13 patients had an aspiration biopsy of bone marrow during the episodes of MAS and hemophagocytosis occurred in all of them (Table 2).

Genotyping results for perforin A91V polymorphism

PCR amplified DNA fragments containing the A91V polymorphism position in exon 2 of the perforin gene. The fragment size was 171 bp (Fig.). R was detected in the single nucleotide polymorphisms (SNPs) position in exon 2 of the perforin gene. The genotyping of 6 SoJIA patients with MAS were all wild-type with no mutation.

Treatment and outcome

Corticosteroids were considered the first line treatment for the patients. Dexamethasone as a steroid source was administered alone in 6 patients, dexamethasone plus VCR in 3 patients, dexamethason plus VP16 in 2 patients, dexamethasone plus VCR and VP16 in one patient, and dexamethasone plus cyclosporin A, gammaglobulin and VP16 in one patient. One patient received plasmapheresis. Two patients underwent artificial ventilation. Since the patients with MAS did not respond to antibiotics, the treatment with antibiotics was not adopted except for those with invasive infection. After treatment, 10 patients recovered completely and 3 died.

Discussion

The term MAS has been used almost exclusively to describe this condition in association with rheumatic diseases. It is most commonly seen with systemic onset juvenile rheumatoid arthritis (JRA) and is also

Fig. A 171-bp fragment of the perforin gene including the second extron being amplified by PCR.

associated with systemic lupus erythematosus, juvenile dermatomyositis, and Kawasaki disease. In 1985, Hadchouel et al[5] described 7 patients with systemic onset of JRA who developed this complication during the course of their disease. In 1993, the term MAS was used to define this clinical manifestation, and since then, MAS has been widely used in rheumatic diseases, especially in SoJIA.[6] In our study, all patients were diagnosed as having SoJIA complicated by MAS. Clinicians have suggested MAS is HLH related rheumatic diseases. Although MAS bears clinical and biological similarities to other rheumatic diseases mentioned above, it is unique in three distinct ways.[2] First, MAS appears to occur with varying degrees of severity, ranging from the moribund child with persistent high grade fever, significant hepatosplenomegaly, icterus, and laboratory parameters showing pancytopenia, coagulopathy, hepatic, and renal disorders to the unwell child with persistent fever, no significant organomegaly, a relative drop in blood cell counting, and mild if any coagulopathy. The biology behind these varied changes is poorly understood. Second, MAS occurs in systemic rheumatic diseases, primarily SoJIA, which is an inflammatory disorder with hematological abnormalities reflecting this. Thus, anemia, leucocytosis, and thrombocytosis with a raised ESR and C reactive protein are typical indexes of active disease. A significant drop in these laboratory parameters, even though technically not in the range of thrombocytopenia or leucopenia, cannot be dismissed as irrelevants. It may well represent the onset of MAS in the correct clinical setting, as was seen in our patients. A "relative" change in counts may in fact be a key to early diagnosis and appropriate intervention. Third, early and aggressive immunosuppression is likely to benefit these patients, unlike in familial HLH where bone marrow transplantation is the cure.

Most of our patients were typical. But in others, some features were not obvious because cytopenia, hyperglycemia and hypofibrinogenenia were related to the severity of the involved organs. Other symptoms such as splenomegaly only occurred in the later stage in some of our patients. We therefore emphasized that HLH should not be excluded for some clinical features not meeting the HLH criteria, and that repeated examinations were necessary in some suspicious patients. Otherwise, some patients in the early stage would be excluded. Hemophagocytosis is an important index in the diagnosis of MAS. In MAS series, hemophagocytosis can be found only in later stage in a proportion of patients. Marrow aspiration biopsy has a lower positive rate and repeated marrow aspiration biopsy is required. Although biopsy of the spleen and lymph nodes has a higher positive rate, it is difficult in
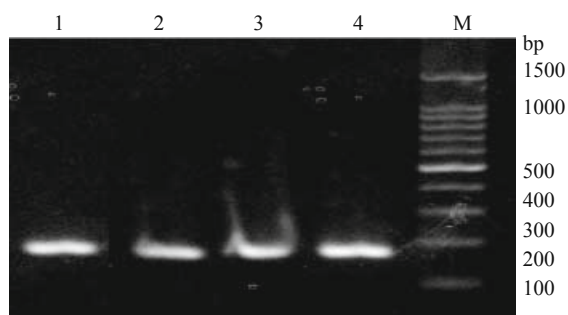
practice especially in patients with DIC. Thus negative hemophagocytosis can not rule out HLH. Serum ferritin and lactate dehydrogenase (LDH) are not included in the 1991 HLH criteria, but more attention should be paid to their laboratory examinations. In a study of secondary HLH, hyperferritinemia (ferritin >1000 μg/L) was found in 90% and elevated LDH (LDH >1000 iu/L) in 89.7% of patients whereas hyperglycemia and hypofibrinogenesis in 50% and 57.4%, respectively.[7] Despite their poor specificies, serum ferritin and LDH are sensitive indexes for the diagnosis of HLH. In our study, cases of hyperferritinemia and cases of elevated LDH accounted for 55.5% and 88.9% respectively. Hence, the new 2004 criteria set forth by the International Histocyte Institute included 3 new indexes: decreased or lack of natural killer (NK) cell activities, hyperferritinemia, and evaluated soluble CD25 (>2400 U/ml).[8] In China, however, the 1991 HLH criteria are still widely used. The detection of NK cell activity can help to differentiate primary from secondary HLH. In the primary HLH, the decrease of NK cell activity is prolonged. But in the secondary HLH, the abnormal condition will disappear soon after the recovery of the patient.[9-11]

Triggers of MAS can be divided into two types: infection factor and pharmaceutical factor.[12] Infection factors include bacteria, virus, parasite and fungi. Pharmaceutical factors include non-steroid anti-inflammatory agents (e.g., aspirin), anti-epileptic drugs, gold preparation sulfasalazine, MTX, parenteral nutrition, TNF-α antagon, and CD52 antagon. In our study, viral infection occurred in 3 patients. Drugs as triggers were not found in our study. Recent studies have indicated that the mechanism of MAS might be related to cytokines caused by the abnormal expression of the perforin gene and functional disorder of NK cells.[13-18] Abnormal function of cytotoxic lytic activities of NK cells of SoJRA with MAS was reported to be associated with decreased perforin expression and the pathogenesis of the syndrome. Abnormal cytotoxic cells might fail to provide appropriate apoptotic signals for the removal of activated macrophages after infection is cleared. These immunologic abnormalities are due to mutations in the gene encoding perforin, a protein that mediates the cytotoxic activity of NK.[19] A91V is the most common amino-acid substitution identified in perforin, with an allele frequency ranging between 3% and 17% in the general population.[20] But in our study, the perforin A91V (NCBI:SNP rs35947132) gene in 6 SoJIA patients with MAS were normal. MAS has been reported to respond to corticosteroids alone or in appropriate supportive management. In a recent case series, remission was induced in 15 of the 21 episodes of MAS by steroids alone. Patients with a suboptimal

response to corticosteroids have been well with the addition of cyclosporin A.[7] Only a few reports focused on patients with MAS who were treated with other agents including etoposide. Plasmapheresis was also effective in some cases.[21] Mouy et al[22] described that 5 cases (4 SoJIA and 1 polyarthritis type JIA) responded to cyclosporin A effectively. Kounami et al[23] held that cyclosporin A may be the choice of first-line treatment. In our study, administration of corticosteroids along with VCR and/or VP16 worked effectively. In an episode, the child did not respond to the therapy with dexamethasone plus cyclosporin A and gammaglobulin, but after the combined administration with VP16, the patient recovered rapidly. In another patient, ARDS, MODS and pneumonorrhagia occurred during the episode, and plasmapheresis together with pharmaceutical treatment helped the patient to pull through. Thus the agents for the treatment of patients consist of corticosteroids, cyclosporin A, plasmapheresis, and gammaglobulin. Once MAS occurs, the patients' condition will deteriorate rapidly. Therefore, prompt diagnosis and treatment of MAS are essentially important to a better outcome.

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