Differentiating Macrophage Activation Syndrome in Systemic Juvenile Idiopathic Arthritis from Other Forms of Hemophagocytic Lymphohistiocytosis

Kai Lehmberg, MD[1,*], Isabell Pink[1,*], Christine Eulenburg, PhD[2], Karin Beutel, MD[3], Andrea Maul-Pavicic, PhD[4], and Gritta Janka, MD[1]

Objectives To identify measures distinguishing macrophage activation syndrome (MAS) in systemic juvenile idiopathic arthritis (sJIA) from familial hemophagocytic lymphohistiocytosis (FHL) and virus-associated hemophagocytic lymphohistiocytosis (VA-HLH) and to define appropriate cutoff values. To evaluate suggested dynamic measures differentiating MAS in patients with sJIA from sJIA flares.

Study design In a cohort of patients referred for evaluation of hemophagocytic lymphohistiocytosis, we identified 27 patients with sJIA and MAS (MAS/sJIA) fulfilling the criteria of the proposed preliminary diagnostic guideline for the diagnosis of MAS in sJIA. Ten measures at diagnosis were compared between the MAS/sJIA group and 90 patients with FHL and 42 patients with VA-HLH, and cutoff values were determined. In addition, 5 measures were analyzed for significant change from before MAS until MAS diagnosis.

Results Neutrophil count and C-reactive protein were significantly higher in patients with MAS/sJIA compared with patients with FHL and patients with VA-HLH, with 1.8 $\times$ 10^9/L neutrophils (sensitivity 85%, specificity 83%) and 90 mg/L C-reactive protein (74%, 89%) as cutoff values. Soluble CD25 <7900 U/L (79%, 76%) indicated MAS/sJIA rather than FHL/VA-HLH. Platelet (−59%) and white blood cell count (−46%) displayed a significant decrease, and neutrophil count (−35%) and fibrinogen (−28%) showed a trend during the development of MAS. However, a substantial portion of patients had values at diagnosis of MAS within or above the normal range for white blood cells (84%), neutrophils (77%), platelets (26%), and fibrinogen (71%).

Conclusion Readily available measures can rapidly differentiate between MAS/sJIA and FHL/VA-HLH. The findings substantiate that a decline of measures may facilitate the distinction of MAS from flares of sJIA. *(J Pediatr 2013;162:1245-51)*.

Acquired hemophagocytic lymphohistiocytosis (HLH) is a severe complication of autoimmune and autoinflammatory disease. By convention, this type of HLH is termed macrophage activation syndrome (MAS).[1] It is commonly associated with systemic juvenile idiopathic arthritis (sJIA) but also has been described in systemic lupus erythematosus,[2] inflammatory bowel disease,[3] and Kawasaki disease.[4] The condition is potentially fatal[5] and requires rapid recognition to initiate prompt treatment and prevent deleterious outcome. However, particularly in sJIA, the diagnosis is frequently difficult to make, and there is no gold standard to identify the condition with high sensitivity and specificity.

MAS can be the initial disease presentation of sJIA. In these cases, clinical signs such as arthritis frequently appear only later. This renders the distinction between MAS and hereditary HLH or acquired virus-associated HLH (VA-HLH) particularly difficult. Due to recent advances in the description of HLH-related gene defects, most patients with hereditary HLH can be identified through genetic analysis.[6] Flow cytometric analyses allow detection of a hereditary form in most cases within 48-72 hours. Intracellular perforin, SLAM-associated protein, and x-linked inhibitor of apoptosis can be stained, and reduced or absent appearance of CD107 on the surface of natural killer (NK) cells after stimulation indicates genetic defects of lytic granule transport and release (CD107 degranulation assay).[7] However, these investigations are not always easily available. Furthermore, the presence of a viral infection such as Epstein-Barr virus (EBV) and cytomegalovirus in a patient with HLH does not allow classification of the disease as VA-HLH. Viral infections not only can trigger HLH in otherwise healthy individuals but also can set

AUC	Area under the curve	PDG	Preliminary diagnostic guideline
CRP	C-reactive protein	ROC	Receiver operating characteristic
EBV	Epstein-Barr virus	sCD	Soluble CD
FHL	Familial hemophagocytic lymphohistiocytosis	sJIA	Systemic juvenile idiopathic arthritis
HLH	Hemophagocytic lymphohistiocytosis	VA-HLH	Virus-associated hemophagocytic lymphohistiocytosis
MAS	Macrophage activation syndrome	WBC	White blood cell
NK	Natural killer		

From the Departments of [1]Pediatric Hematology and Oncology, [2]Medical Biometry and Epidemiology, University Medical Center Eppendorf; [3]Department of Pediatric Hematology and Oncology, University Hospital Münster, Germany; and [4]Center of Chronic Immunodeficiency, University Medical Center, Freiburg, Germany

*Contributed equally.

The authors declare no conflicts of interest.

0022-3476/$ - see front matter. http://dx.doi.org/10.1016/j.jpeds.2012.11.081

off or exacerbate an episode in patients with genetic disease predisposing to HLH or with MAS in autoimmune and autoinflammatory conditions.[8,9] In this study, we established routine laboratory measures that help to distinguish sJIA-associated MAS from familial HLH (FHL) and VA-HLH.

Another clinical challenge is the proper distinction between a flare of sJIA and the development of sJIA-associated MAS, because the 2 conditions have overlapping features. For HLH, the HLH-2004 criteria have been established by the Histiocyte Society.[10] However, patients with autoinflammatory disease including sJIA usually display high white blood cell (WBC), neutrophil, and platelet counts and fibrinogen levels, as a feature of disease activity. A drop of these measures from elevated to normal values in an ongoing disease flare may indicate MAS,[9] which will not be interpreted correctly when using the HLH-2004 criteria. To address this problem, a preliminary diagnostic guideline (PDG) for MAS was suggested, based on the analysis of a cohort of patients with MAS compared with a group of patients with an acute flare of sJIA.[11] According to the PDG, the diagnosis of MAS requires the presence of ≥2 laboratory criteria or of ≥2 clinical and/or laboratory criteria: platelet count $<262 \times 10^9$/L, aspartate aminotransferase >59 U/L, WBC $<4 \times 10^9$/L, fibrinogen <2.5 g/L, hepatomegaly, neurologic symptoms, and hemorrhages. In this study, the fulfillment of the PDG criteria was mandatory for inclusion in the cohort of patients with MAS. We corroborated the preliminary findings of others[9,12,13] that the dynamics of measures during the development of MAS can help to differentiate MAS from flares of sJIA.

Methods

Patient data were retrieved from the database of the German national HLH study center, to which patients are referred from Germany, Austria, and Switzerland. Patients with rheumatic disease are not routinely reported, but patients are registered if the center is contacted due to uncertainty about the diagnosis and/or treatment of MAS or if MAS is the presenting feature of a condition, such as sJIA. Diagnosis of MAS or HLH was made between 1992 and 2010. Data were obtained at diagnosis before the start of immunosuppressive therapy for MAS or HLH. In cases of known sJIA with continuous immunomodulatory therapy, data were recorded before MAS-directed therapy was commenced.

Forty-seven patients <18 years of age with suspected MAS in sJIA were identified (MAS/sJIA group). We excluded 17 patients who on revision did not fulfill the revised International League of Associations for Rheumatism criteria for sJIA,[12] 1 patient who did not fulfill the PDG criteria,[11] and 2 patients for whom data were insufficient. Overall, 27 patients were included in the analysis. Studies HLH-94 and HLH-2004 were approved by the Ethics Committee of the Hamburg Chamber of Physicians; informed consent was obtained from the legal guardians.

In 90 patients with a genetic diagnosis of FHL, data obtained at diagnosis of HLH fulfilling the HLH-2004 criteria[10] were analyzed as controls (FHL group). In addition, 42 patients with VA-HLH (VA-HLH group) were studied, defined by fulfillment of the HLH-2004 criteria,[10] detection of EBV, cytomegalovirus, parvovirus B19, human herpes virus 6, or adenovirus by polymerase chain reaction or unequivocal serological analysis, and absence of relapse for >1 year. Patients with a genetic disorder predisposing to HLH, parental consanguinity, familial disease, abnormal perforin expression or abnormal degranulation of NK cells, signs of autoimmune or autoinflammatory disease, or *Leishmania* infection were excluded in the VA-HLH group.

Statistical Analyses

In a first step, the natural logarithms of the mean of hemoglobin level; WBC, neutrophil, and platelet counts; serum ferritin; fibrinogen; triglycerides; soluble CD (sCD)25 (Immulite immunoassay system, Siemens Healthcare Diagnostics, New York, New York); and C-reactive protein (CRP) at diagnosis of MAS or HLH were compared between (1) MAS/sJIA and FHL and (2) MAS/sJIA and VA-HLH using a 1-way ANCOVA with adjustment for age at onset and post-hoc testing. In addition, this step was performed for age at onset using ANOVA. Laboratory measures of FHL and VA-HLH were in a similar range. Thus, in a second step, FHL and VA-HLH laboratory data were pooled (FHL/VA-HLH) and a comparison was performed (3) between MAS/sJIA and FHL/VA-HLH using a 1-way ANCOVA with adjustment for age at onset. Differences were considered significant at $P < .0017$ after Bonferroni adjustment for 30 comparisons corresponding to an overall level of significance of .05. We tested for sex as a confounding variable by including this factor as an adjusting covariable in a first step. Because sex had no significant impact on the results, this factor was eliminated for further conclusions (backward selection). Finally, receiver operating characteristic (ROC) curves were calculated to determine cutoff values for the differentiation between MAS/sJIA and FHL/VA-HLH. Values for which the sum of sensitivity and specificity reached the maximum were taken as cutoffs. Measures that did not allow identification of 1 single cutoff value with high separation accuracy were excluded.

In addition, we calculated the change in WBC, neutrophil, and platelet counts, fibrinogen, and CRP. To this end, data for our patients were analyzed at 2 time points: before the development of MAS and at diagnosis before the start of MAS-directed therapy. From each patient, the only data included were those for which both values were available. The overall difference was described by the median of the proportionate change of each patient. Significance of differences was assumed at $P < .01$, as determined with a 2-sided Student t test for matched pairs after Bonferroni adjustment for 5 comparisons, corresponding to an overall level of significance of .05. To compare the relevance of the dynamic measures to absolute figures, the number of patients with WBC, neutrophil, and platelet counts and fibrinogen at diagnosis within or above the normal range was determined (including only those data points that had been used previously in the evaluation of the dynamic measures).