Development and Initial Validation of the Macrophage Activation Syndrome/Primary Hemophagocytic Lymphohistiocytosis Score, a Diagnostic Tool that Differentiates Primary Hemophagocytic Lymphohistiocytosis from Macrophage Activation Syndrome

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Objective To develop and validate a diagnostic score that assists in discriminating primary hemophagocytic lymphohistiocytosis (pHLH) from macrophage activation syndrome (MAS) related to systemic juvenile idiopathic arthritis.

Study design The clinical, laboratory, and histopathologic features of 362 patients with MAS and 258 patients with pHLH were collected in a multinational collaborative study. Eighty percent of the population was assessed to develop the score and the remaining 20% constituted the validation sample. Variables that entered the best fitted model of logistic regression were assigned a score, based on their statistical weight. The MAS/HLH (MH) score was made up with the individual scores of selected variables. The cutoff in the MH score that discriminated pHLH from MAS best was calculated by means of receiver operating characteristic curve analysis. Score performance was examined in both developmental and validation samples.

Results Six variables composed the MH score: age at onset, neutrophil count, fibrinogen, splenomegaly, platelet count, and hemoglobin. The MH score ranged from 0 to 123, and its median value was 97 (1st-3rd quartile 75-123) and 12 (1st-3rd quartile 11-34) in pHLH and MAS, respectively. The probability of a diagnosis of pHLH ranged from <1% for a score of <11 to >99% for a score of ≥123. A cutoff value of ≥60 revealed the best performance in discriminating pHLH from MAS.

Conclusion The MH score is a powerful tool that may aid practitioners to identify patients who are more likely to have pHLH and, thus, could be prioritized for functional and genetic testing. *(J Pediatr 2017;189:72-8).*

See editorial, p 19

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome caused by a severely dysregulated immune response.[1] It is characterized by highly activated lymphocytes and macrophages that infiltrate tissues and produce large amounts of proinflammatory cytokines.[2,3] A set of clinical, laboratory, and histopathologic features define the acute syndrome, including unremitting fever, hepatosplenomegaly, cytopenia, hypofibrinogenemia, elevated ferritin, liver enzymes, triglycerides, and soluble CD25 (or soluble interleukin-2 receptor α chain), and hemophagocytosis (the engulfment of blood cells by activated macrophages) in different tissues and organs.[4]

HLH	Hemophagocytic lymphohistiocytosis
MAS	Macrophage activation syndrome
pHLH	Primary hemophagocytic lymphohistiocytosis
ROC	Receiver operator characteristic
sJIA	Systemic juvenile idiopathic arthritis

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HLH comprises a heterogeneous spectrum of clinically similar but etiologically diverse subtypes, affecting all ages. In the current classification of histiocytic disorders, it is subdivided into primary and secondary forms.[5-7] Primary HLH (pHLH, also called familial HLH) refers to cases associated with several inherited monogenic disorders.[8] Secondary HLH (also known as acquired or reactive HLH) is not typically inherited, but complicates various medical conditions, including infections, malignancies, and rheumatic diseases. By convention, secondary HLH seen in rheumatic disorders is termed macrophage activation syndrome (MAS).[9] In childhood, this condition occurs most commonly in systemic juvenile idiopathic arthritis (sJIA).[10-12]

Both pHLH and MAS are potentially fatal and require immediate recognition to initiate prompt treatment and avoid a deleterious outcome. However, because they bear close clinical similarities, their differentiation may be challenging. Distinction may be particularly difficult when MAS is the initial clinical presentation of sJIA and arthritis is not yet present. Diagnostic challenges are compounded by the increasing number of reports of patients who develop pHLH in adolescence or adulthood.[13] Indeed, although pHLH typically develops in the first year of life, it is now understood that there are patients with a genetic basis for this illness who remain asymptomatic until a late age.[14] Documentation of biallelic pathologic mutations in a disease-associated gene is the gold standard diagnostic test for pHLH. However, these studies take weeks to complete and may not be available in resource-limited areas. In addition, although most cases of pHLH can be verified molecularly, some cases still elude molecular diagnosis.[15] Moreover, interpretation of genetic studies is not always straightforward because some genetic overlap between MAS and pHLH has been identified.[16,17] Indeed, a substantial percentage of patients with secondary HLH, including MAS, possess heterozygous mutations in the same perforin-mediated cytolytic pathway genes associated with pHLH.[18-20] Recently, some of these heterozygous mutations have been shown to contribute to disease pathophysiology by acting as dominant-negative mutants.[21,22]

Timely diagnosis is essential, because pHLH is often more severe than MAS. In addition, management of the 2 conditions differs. Although both are treated with intravenous corticosteroids and cyclosporine, the treatment protocols recommended for pHLH (HLH-94) involves dexamethasone and etoposide,[23] whereas pediatric rheumatologists almost always use in MAS higher corticosteroid equivalents of methylprednisolone and interleukin-1 blockers.[10] Furthermore, patients with pHLH often require allogeneic hematopoietic stem cell transplantation sooner than later.[24] In contrast, patients with sJIA almost never need such treatment.

Diagnostic or classification criteria are available for both pHLH[7] and MAS.[25-27] However, their ability to discriminate between the 2 syndromes has not been investigated previously. Recently, Fardet et al[28] published a weighted diagnostic score for the broader category of reactive hemophagocytic syndrome, called the HScore. However, because their patient sample was only composed of adults with reactive hemophagocytic syndrome, no information can be drawn reliably regarding the applicability of the HScore to pediatric patients with pHLH or MAS. Moreover, it has been argued that the use in MAS of some individual criteria included in the HScore may be problematic.[29]

The primary purpose of this international collaborative project was to develop and validate a diagnostic score that assists in discriminating pHLH from sJIA-associated MAS.

Methods

Data from patients with MAS were collected in the context of the multinational collaborative effort that led to the development of the 2016 classification criteria for MAS complicating sJIA.[26,27] The design, inclusion criteria, and data collection procedures of this project have been described in detail elsewhere.[26,27,30-32] Briefly, international pediatric rheumatologists and pediatric hematologists were asked to collect data from patients with sJIA-associated MAS seen at their institution after 2002. To be included in the study, patients had to have sJIA[33] and to have had an episode of MAS diagnosed and treated as such by the caring physician.

Data from patients with pHLH were retrieved from both the HLH-94[23] and HLH-2004[7] trials and were recorded in the Histiocyte Society Register. The diagnosis of pHLH was based on the HLH-94 or HLH-2004 criteria, depending on the year of observation. All patients had their diagnosis confirmed genetically. For all patients with MAS or pHLH, demographic, clinical, laboratory, and histopathologic information was collected at disease onset. The study protocol was approved by the ethics committee at each participating center.

Statistical Analyses

The methodology used for the construction of the MAS/HLH (MH) score shares many features with that used by Fardet et al[28] for the development of the HS score. Eighty percent of patients enrolled in the study were assigned randomly to the developmental dataset by stratifying MAS and patients with pHLH, and the remaining 20% were assigned to the validation dataset. The developmental and validation groups were comparable for all clinical, laboratory, and histopathologic characteristics (data not shown). The features of patients with pHLH and MAS were compared by the χ^2 test (for categorical variables) and Mann-Whitney *U* test (for continuous variables).

To enhance the feasibility of the score, before inclusion in univariate analysis, continuous variables were dichotomized through a receiver operator characteristic (ROC) curve analysis, retaining the value at which sensitivity and specificity were maximized. All variables listed in **Table I** were assessed. The variables with the strongest association with the diagnosis of pHLH (ie, those that achieved an OR > 5 and a $P < .05$) were scrutinized further on multivariable logistic regression procedures to evaluate their independent contribution to the outcome (ie, the diagnosis of pHLH). The characteristics of patients with missing variables were compared with those of