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Development and Validation of the HScore, a Score for the Diagnosis of Reactive Hemophagocytic Syndrome

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Objective. **Because it has no unique clinical, biologic, or histologic features, reactive hemophagocytic syndrome may be difficult to distinguish from other diseases such as severe sepsis or hematologic malignancies. This study was undertaken to develop and validate a diagnostic score for reactive hemophagocytic syndrome.**

Methods. **A multicenter retrospective cohort of 312 patients who were judged by experts to have reactive hemophagocytic syndrome (n = 162), were judged by experts to not have reactive hemophagocytic syndrome (n = 104), or in whom the diagnosis of reactive hemophagocytic syndrome was undetermined (n = 46) was used to construct and validate the reactive hemophagocytic syndrome diagnostic score, called the HScore. Ten explanatory variables were evaluated for their association with the diagnosis of hemophagocytic syndrome, and logistic regression was used to calculate the weight of each criterion included in the score. Performance of the score was assessed using developmental and validation data sets.**

Results. **Nine variables (3 clinical [i.e., known underlying immunosuppression, high temperature, organomegaly], 5 biologic [i.e., triglyceride, ferritin, serum glutamic oxaloacetic transaminase, and fibrinogen levels, cytopenia], and 1 cytologic [i.e., hemophagocytosis features on bone marrow aspirate]) were retained in the HScore. The possible number of points assigned to each variable ranged from 0–18 for known underlying immunosuppression to 0–64 for triglyceride level. The median HScore was 230 (interquartile range [IQR] 203–257) for patients with a positive diagnosis of reactive hemophagocytic syndrome and 125 (IQR 91–150) for patients with a negative diagnosis. The probability of having hemophagocytic syndrome ranged from <1% with an HScore of ≤90 to >99% with an HScore of ≥250.**

Conclusion. **The HScore can be used to estimate an individual's risk of having reactive hemophagocytic syndrome. This scoring system is freely available online (http://saintantoine.aphp.fr/score/).**

Hemophagocytic syndrome is a hyperinflammatory condition caused by highly stimulated but dysregulated and often ineffective immune responses. Its cardinal features are fever, hepatosplenomegaly, pancytopenia, and widespread histiocytic tissue infiltration. There are 2 major forms of hemophagocytic syndrome: a primary (hereditary) form which occurs in early childhood, and a secondary (reactive) form which occurs at any age and is probably much more frequent than the primary form (1–5).

Reactive hemophagocytic syndrome may be related to infection, malignancy, or autoimmune disease (6–10), mainly in the setting of underlying immunosuppression. It is a severe, life-threatening condition with a

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mortality rate ranging from 10% in children with systemic juvenile idiopathic arthritis to 20–60% in adults with malignancy-associated hemophagocytic syndrome (4,11). Timely diagnosis is essential since early administration of effective therapy (e.g., etoposide) may improve survival (12). However, diagnosis of hemophagocytic syndrome is difficult since there are no unique clinical, biologic, or pathologic features. Bone marrow hemophagocytosis may be observed in the absence of proven hemophagocytic syndrome, in particular after blood transfusion or in critically ill patients with sepsis (13–15). Clinical features (e.g., fever, hepatosplenomegaly) and biologic features (e.g., cytopenia, hyperferritinemia) observed in patients with hemophagocytic syndrome also occur in other disorders, and the syndrome may be difficult to distinguish from other diseases such as severe sepsis or hematologic malignancies.

Diagnostic criteria sets for reactive hemophagocytic syndrome have been proposed (1,8,16–19), but they suffer from substantial limitations. First, some have been proposed specifically for pediatric populations or to diagnose the primary form of the syndrome, which is mostly observed in the context of hereditary disease. They have never been validated in adults or in the reactive form of the syndrome. Second, the weight of each criterion included in these scoring systems is unknown, and the proposed cutoff values were empirically defined. Third, some of the proposed criteria (e.g., natural killer cell activity, soluble interleukin-2 receptor level) cannot be measured in routine practice and may be of low interest for the diagnosis of the reactive form of the syndrome. The aim of the present study was to construct and validate a set of weighted criteria, called the HScore, for the diagnosis of reactive hemophagocytic syndrome.

PATIENTS AND METHODS

Patients. Between June and November 2012, we retrospectively reviewed all of the forms for and results of bone marrow aspirations performed between January 2006 and December 2011 at 3 university-affiliated tertiary hospitals in France. First, all forms containing orders for bone marrow aspiration for suspected hemophagocytic syndrome and all bone marrow aspirations that led to a diagnosis of hemophagocytosis were identified. Next, we identified all patients in these centers who had International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) codes of D76.1 (hemophagocytic lymphohistiocytosis), D76.2 (hemophagocytic syndrome, infection-associated), or D76.3 (other histiocytosis syndromes) during the study period. The 2 resulting lists of patients were cross-referenced in order to ensure that no patient with a code for hemophagocytic syndrome who had undergone bone marrow aspiration was missed.

We then retrospectively reviewed the medical records of all of the patients identified. Medical information was extracted from the records using a standardized questionnaire that addressed demographic characteristics (age, sex), clinical features (highest recorded temperature, duration of fever [if any], presence of hepato-, spleno-, or adenomegaly, earlier medical history, known underlying immunosuppression (i.e., presence of human immunodeficiency virus or long-term treatment with an immunosuppressive agent such as glucocorticoids, cyclosporine, or azathioprine], diagnosis of hemophagocytic syndrome retained or not by the medical team caring for the patient, treatment prescribed, underlying disease, transfer to intensive care unit, outcome), laboratory findings (leukocyte and platelet counts, hemoglobin, liver enzyme, ferritin, triglyceride, cholesterol, fibrinogen, C-reactive protein, lactate dehydrogenase [LDH], blood urea nitrogen, creatinine, and sodium levels, prothrombin time), and pathologic findings (hemophagocytosis on bone marrow aspiration or biopsy). The laboratory parameters extracted from the medical records were those obtained on the day of bone marrow aspiration, or those obtained up to 2 days before or after in the absence of data from the simultaneous day. Only the first episode was considered in patients with recurrent hemophagocytic syndrome.

Classification procedure. In a first step, 3 of the authors (LF, LG, PC) classified cases into 3 groups: hemophagocytic syndrome likely (positive cases), hemophagocytic syndrome possible (undetermined cases), or hemophagocytic syndrome unlikely (negative cases). Classification was based on information extracted from the patient's medical record. The investigators classifying the patients had access to followup data and information about the underlying disease. Each investigator classified patients without knowledge of the classifications made by the others or of the results of a Delphi study conducted at the same time and described elsewhere (20). After all of the patients were classified by all 3 investigators, the results were compared. Positive/undetermined and undetermined/negative classifications were considered as minor discordances, whereas positive/negative classifications were considered as major discordances. All instances of minor discordance were discussed among the 3 investigators, and a consensus was reached in many cases. In cases where a consensus was not reached or initial classification led to major discordances, a fourth expert (OL) was engaged and a final classification was made when 3 of the 4 experts were in agreement, the remaining cases being classified as undetermined. Each of the 4 experts involved in this classification procedure had at least 10 years of experience in diagnosing and caring for patients with reactive hemophagocytic syndrome.

Variables of interest. Ten explanatory variables were evaluated for their association with the diagnosis of hemophagocytic syndrome. These 10 variables were those issued from a Delphi consensus study described elsewhere (20). In summary, 63 international experts in reactive hemophagocytic syndrome were solicited between October and December 2012, and 24 experts from 13 countries participated in the second and final Delphi round. The questionnaire addressed their opinions regarding the helpfulness of a predefined list of criteria for a positive diagnosis of reactive hemophagocytic

syndrome. This Delphi survey showed that a positive consensus (i.e., criteria considered by the experts as "absolutely required" or "important") was reached for the following 7 criteria: cytopenia (involving cells of 1 lineage, cells of 2 lineages, or pancytopenia), presence of hemophagocytosis documented by bone marrow aspiration or biopsy, fever, organomegaly, presence of a predisposing underlying disease, high levels of LDH, and high levels of ferritin. A negative consensus was reached for 13 criteria. No consensus was reached for 4 criteria (i.e., high transaminase levels, high triglyceride levels, low fibrinogen levels, and low glycosylated ferritin levels). In the present study, we included all 7 positive criteria listed above, plus 3 of the 4 criteria for which no consensus was reached. (We chose not to include the percentage of glycosylated ferritin as this parameter is very rarely assessed in clinical practice.)

Statistical analysis. Construction of the HScore shares many methodologic features with those used in the previously described construction and validation of the Simplified Acute Physiology Score II score (21). To develop the diagnostic score, undetermined cases were excluded. Of the cases classified as positive or negative, 90% were randomly assigned to the developmental data set, while the remaining 10% were assigned to the validation data set. The group of cases classified as positive and the group classified as negative were compared by Fisher's exact test (for categorical variables) and Wilcoxon's rank sum test (for continuous variables). By univariate analysis, each of the 10 variables of interest was associated with the positive diagnosis of reactive hemophagocytic syndrome, with a P value of <0.05. They were therefore included in a multivariate logistic model to assess their independent contribution to the outcome. Binary variables included in the model (e.g., hemophagocytosis features seen on bone marrow aspirate) were coded as present or absent. For continuous variables, linearity was assessed using the log-likelihood ratio test. In order to obtain a score that would be easy to apply, variables showing a linear relationship with the outcome were dichotomized. The threshold value was based on a receiver operating characteristic (ROC) curve analysis, retaining the value at which sensitivity plus specificity was maximized. For variables not showing a linear relationship with the outcome, the lowess smoothing function was used to suggest ranges for each variable, and the resulting plot was examined to identify cutoff values. No interaction terms were included in the model. The pseudo R^2 statistic was used for assessing the goodness-of-fit of the model.

The coefficients resulting from this multiple logistic regression analysis were used to assign score points for construction of the HScore. For each variable that was significantly associated with the outcome in the logistic regression, the rule was to multiply the beta value for each range by 10 and round off to the nearest integer. Once the score was calculated for each case from the developmental data set, it was used in another multiple logistic regression equation designed to be converted to a probability of having hemophagocytic syndrome. An equation based on this multiple logistic regression model was developed. The first step was to compute the logit, as follows: $\text{logit} = \beta_0 + (\beta_1 \times \text{HScore})$, after verifying that the HScore was normally distributed. The second step was to convert this logit to a probability (Pr) of hemophagocytic syndrome, with the following equation: $\Pr(y = 1/\text{logit}) = e^{\text{logit}}/(1 + e^{\text{logit}})$. Finally, to assess the performance of the system, Hosmer-Lemeshow tests were performed on both the developmental and the validation sets to evaluate calibration (22), and areas under the ROC curves were used to evaluate discrimination.

Continuous variables are presented as median and interquartile ranges (IQRs). Categorical variables are presented as proportions. Missing data were not imputed or included in the analyses. All analyses were performed using Stata, version 11.1 (StataCorp, College Station, TX).

RESULTS

Study population. Of the 314 patients identified, 2 were excluded because of insufficient available data for classification. Of the remaining 312 patients, 196 (63%) were male, and the median age was 51 years (IQR 36–64) (Table 1). The 3 investigators initially classifying these patients with regard to the diagnosis of hemophagocytic syndrome reached perfect consensus in 179 cases (57%). There were minor discordances in the initial classification of 96 cases (31%, with 78 cases reconciled after discussion) and major discordances in the initial classification of 37 cases (12%). The fourth expert opinion was therefore required for 55 cases (18%). At the end of the process, agreement regarding the presence or absence of hemophagocytic syndrome (i.e., same classification by at least 3 experts) was reached for 304 of the 312 cases, with the 8 remaining cases therefore being classified as undetermined.

Variables of interest (see Patients and Methods) in the 162 patients classified as having a positive diagnosis of hemophagocytic syndrome (52%), the 104 classified as having a negative diagnosis of hemophagocytic syndrome (33%), and the 46 for whom the diagnosis of hemophagocytic syndrome was classified as undetermined (15%) (the above-mentioned 8 patients for whom there was lack of agreement by the experts, and 38 patients for whom the experts agreed that "undetermined" was the appropriate classification) are reported in Table 2. The parameters for which data were most frequently missing were ferritin levels (data missing for 14% of the patients) and triglyceride levels (data missing for 9% of the patients).

Construction of the HScore. The developmental data set included data on 238 patients, of whom 29 were not included in the multivariate analysis because of missing data on at least 1 parameter. All but 2 patients included in this study had cytopenia involving at least 1 cell type, and we were therefore unable to assess the risk of having hemophagocytic syndrome when no cytopenia

Table 1. Characteristics of the study population classified by the expert investigators as being negative or positive for a diagnosis of hemophagocytic syndrome or as undetermined*

	Negative (n = 104 [33%])	Undetermined (n = 46 [15%])	Positive (n =162 [52%])	*P*, positive vs. negative diagnosis
Male	57 (55)	30 (65)	109 (67)	0.03
Age, median (interquartile range) years	54 (39–65)	55 (37–68)	48 (35–62)	0.02
Underlying disease				
Hematologic malignancy†	28 (27)	14 (30)	92 (57)	
Infection‡	35 (34)	25 (54)	40 (25)	
Hematologic malignancy + infection	1 (1)	0 (0)	6 (4)	
Systemic lupus erythematosus	6 (6)	2 (4)	3 (2)	
Still's disease	1 (1)	1 (2)	2 (1)	
Solid cancer	5 (5)	0 (0)	5 (3)	
Other/unknown	28 (27)	4 (9)	14 (9)	
Diagnosis of hemophagocytic syndrome retained by the medical team caring for the patient	19 (18)	29 (63)	148 (91)	<0.0001

* Except where indicated otherwise, values are the number (%).
† Mainly Hodgkin's lymphoma (n = 27) or non-Hodgkin's lymphoma (n = 84).
‡ Mainly bacteria (n = 51) or mycobacteria (n = 22).

was present. ROC curve analyses resulted in cytopenia being defined as a hemoglobin level of ≤9.2 gm/dl, a leukocyte count of ≤5,000/mm^3, and/or a platelet count of ≤110,000/mm^3. Patients were recorded as having no cytopenia, cytopenia involving 1 of these lineages, cytopenia involving 2 of these lineages, or pancytopenia (all 3 of these lineages). In multivariate analysis, 9 of the 10 variables included in the model remained significantly associated with the probability of being classified as having hemophagocytic syndrome (borderline significant in 1 case [serum glutamic oxaloacetic transaminase]) (Table 3). LDH level was not independently associated with a positive diagnosis of hemophagocytic syndrome ($P = 0.72$ for levels between 500 and 2,000 IU/liter and $P = 0.19$ for levels >2,000 IU/liter, as compared to the reference level of <500 IU/liter). The pseudo R^2 statistic for the model was 0.78 ($P < 0.0001$).

The maximum possible score assigned to each

Table 2. Assessment of the explanatory variables of interest in the study population classified by the expert investigators as being negative or positive for a diagnosis of hemophagocytic syndrome or as undetermined*

	Negative (n = 104 [33%])	Undetermined (n = 46 [15%])	Positive (n =162 [52%])	*P*, positive vs. negative diagnosis
Known immunosuppression, no. (%)†	34 (33)	12 (26)	73 (45)	0.03
Maximal temperature, °C	38.6 (37.9–39.2)	39.2 (38.3–39.8)	39.5 (39.0–40.2)	<0.0001
Hepato- or splenomegaly, no. (%)	23 (22)	12 (26)	28 (17)	<0.0001
Hepato- and splenomegaly, no. (%)	31 (30)	15 (33)	105 (65)	
Hemophagocytosis features on bone marrow aspirate, no. (%)	41 (39)	36 (78)	114 (70)	<0.0001
Leukocyte count, 10^6/liter	4,900 (2,160–10,400)	5,780 (2,200–9,000)	3,100 (1,800–6,500)	0.004
Neutrophils	3,100 (1,260–6,400)	4,625 (1,600–7,910)	2,150 (1,080–4,160)	0.02
Lymphocytes	800 (480–1,300)	690 (350–1,400)	610 (300–1,150)	0.05
Monocytes	310 (150–740)	360 (160–840)	230 (90–440)	0.02
Hemoglobin, gm/dl	9.6 (8.4–10.4)	9.0 (8.4–9.6)	8.3 (7.3–9.2)	<0.0001
Platelets, 10^9/liter	82 (44–196)	54 (29–102)	59 (30–92)	<0.0001
Ferritin, ng/ml	755 (254–1,935)	2,079 (1,350–4,000)	5,139 (2,612–10,000)	<0.0001
Triglycerides, mmoles/liter	1.89 (1.17–2.70)	2.72 (1.78–4.35)	3.06 (2.16–4.18)	<0.0001
Fibrinogen, gm/liter	4.3 (3.3–6.3)	4.4 (3.0–5.9)	3.8 (2.1–5.4)	0.004
LDH, IU/liter	642 (420–933)	660 (377–1,053)	908 (513–1,865)	0.0005
SGOT, IU/liter	44 (26–84)	63 (27–141)	69 (31–171)	0.0004
SGPT, IU/liter	31 (17–64)	36 (16–110)	38 (21–89)	0.14

* Except where indicated otherwise, values are the median (interquartile range). LDH = lactate dehydrogenase; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase.
† Human immunodeficiency virus positive or receiving long-term immunosuppressive therapy (i.e., glucocorticoids, cyclosporine, azathioprine).

Table 3. Variables included in the development of the HScore

	β	SE	P
Known underlying immunosuppression*	1.81	0.86	0.03
Temperature (°C)			
<38.4	1	–	
38.4–39.4	3.35	1.09	0.002
≥39.5	4.89	1.30	<0.0001
Organomegaly			
None	1	–	
Hepatomegaly or splenomegaly	2.33	1.03	0.02
Hepatomegaly and splenomegaly	3.80	1.16	0.001
Cytopenia†			
Cytopenia of 1 lineage	1	–	
Cytopenia of 2 lineages	2.41	1.00	0.02
Cytopenia of 3 lineages	3.36	1.15	0.003
Ferritin (ng/ml)			
<2,000	1	–	
2,000–6,000	3.46	0.94	<0.0001
>6,000	5.01	1.28	<0.0001
Triglyceride (mmoles/liter)			
<1.5	1	–	
1.5–4	4.45	1.44	0.002
>4	6.40	1.74	<0.0001
Fibrinogen (gm/liter)			
>2.5	1	–	
≤2.5	2.96	1.42	0.04
Serum glutamic oxaloacetic transaminase (IU/liter)			
<30	1	–	
≥30	1.86	0.98	0.06
Hemophagocytosis features on bone marrow aspirate	3.49	1.01	<0.0001

* Human immunodeficiency virus positive or receiving long-term immunosuppressive therapy (i.e., glucocorticoids, cyclosporine, azathioprine).
† Defined as a hemoglobin level of ≤9.2 gm/dl and/or a leukocyte count of ≤5,000/mm^3 and/or a platelet count of ≤110,000/mm^3.

variable varied from 18 for underlying immunosuppression to 64 for triglyceride level (Table 4). Missing data were scored as 0. The median HScore was 230 (IQR 203–257) for positive cases and 125 (IQR 91–150) for negative cases. The probability of having hemophagocytic syndrome, by HScore, is shown in Table 5.

Table 5. Probability of hemophagocytic syndrome according to the HScore*

HScore	Probability of hemophagocytic syndrome, %
90	<1
100	1
110	3
120	5
130	9
140	16
150	25
160	40
170	54
180	70
190	80
200	88
210	93
220	96
230	98
240	99
250	>99

* The best cutoff value for HScore was 169, corresponding to a sensitivity of 93%, a specificity of 86%, and accurate classification of 90% of the patients.

Performance of the HScore. In the validation set, the median HScore was 222 (IQR 202–284) for positive cases and 129 (IQR 77–152) for negative cases. The goodness-of-fit test performed on the developmental data set showed a P value of 0.93. When the HScore was applied to the validation data set the P value was 0.76, suggesting that the model can be accurately applied in patients other than those in whom the model was developed. The area under the ROC curve for the HScore was 0.97 and 0.95 in the developmental and

Table 4. The HScore

Parameter	No. of points (criteria for scoring)
Known underlying immunosuppression*	0 (no) or 18 (yes)
Temperature (°C)	0 (<38.4), 33 (38.4–39.4), or 49 (>39.4)
Organomegaly	0 (no), 23 (hepatomegaly or splenomegaly), or 38 (hepatomegaly and splenomegaly)
No. of cytopenias†	0 (1 lineage), 24 (2 lineages), or 34 (3 lineages)
Ferritin (ng/ml)	0 (<2,000), 35 (2,000–6,000), or 50 (>6,000)
Triglyceride (mmoles/liter)	0 (<1.5), 44 (1.5–4), or 64 (>4)
Fibrinogen (gm/liter)	0 (>2.5) or 30 (≤2.5)
Serum glutamic oxaloacetic transaminase (IU/liter)	0 (<30) or 19 (≥30)
Hemophagocytosis features on bone marrow aspirate	0 (no) or 35 (yes)

* Human immunodeficiency virus positive or receiving long-term immunosuppressive therapy (i.e., glucocorticoids, cyclosporine, azathioprine).
† Defined as a hemoglobin level of ≤9.2 gm/dl and/or a leukocyte count of ≤5,000/mm^3 and/or a platelet count of ≤110,000/mm^3.

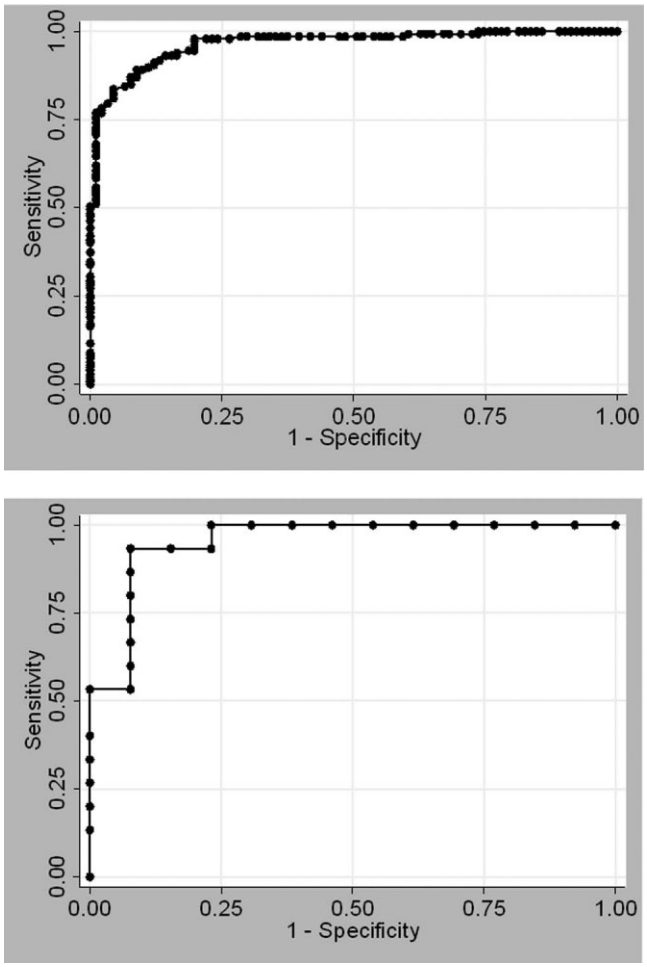

Figure 1. Area under the receiver operating characteristic curve for the HScore in the developmental data set (top) and the validation data set (bottom).

validation data sets, respectively (Figure 1), indicating excellent discrimination

HScore in undetermined cases. The median HScore was 169 (IQR 142–189) in the set of 46 cases for which the presence or absence of hemophagocytic syndrome was classified as undetermined. Of those 46 patients, 11 (24%) had an HScore corresponding to a >80% probability of having hemophagocytic syndrome and 12 (26%) had an HScore corresponding to a probability of <20%.

DISCUSSION

The HScore is the first validated score devoted to the diagnosis of reactive hemophagocytic syndrome. Score construction was based on the largest data set of adult patients with suspected reactive hemophagocytic syndrome reported to date. The score comprised 9 clinical, biologic, and cytologic variables, appropriately weighted.

Hemophagocytic syndrome is a complex disease that may affect a number of different organs, so signs and symptoms may be extremely variable (2,4,23) and no finding is pathognomonic for the disease. In this context, different sets of criteria have been proposed for helping clinicians to diagnose the syndrome (1,8,16–19). However, as described above, existing criteria sets suffer from major limitations. Since hemophagocytic syndrome may be difficult to distinguish from severe sepsis or flare of an underlying disease (e.g., systemic lupus erythematosus, Still's disease, lymphoma) (9,24–26), the availability of a simple score to predict the probability that an individual patient has the syndrome constitutes a major advance, allowing clinicians to make adequate treatment decisions at the earliest opportunity.

Hemophagocytic syndrome is currently considered a rare condition. However, it is likely underdiagnosed due to the lack of defined diagnostic criteria. Among clinicians who are not highly familiar with the syndrome, the presence of hemophagocytosis features on bone marrow aspirate or on biopsy of any other tissue is often thought to be the gold standard for the diagnosis. However, there is no consensus among cytologists regarding which cytologic features are necessary and sufficient for the diagnosis, and in the published literature, cytologic criteria used to define hemophagocytosis are rarely reported (13–15,19). Moreover, features of hemophagocytosis are commonly found in patients who are severely ill with disorders other than hemophagocytic syndrome (13–15). For example, Francois et al reported that 32 of 50 patients (64%) presenting with a sepsis syndrome and thrombocytopenia (<100,000 gm/liter) exhibited hemophagocytosis (13). In another study, hemophagocytosis was evidenced in the bone marrow of 69 of 107 patients (64%) who died in a medical intensive care unit (14). Conversely, initial examination of the bone marrow of patients with authentic hemophagocytic syndrome may reveal only erythroid hyperplasia, leading inexperienced clinicians to wrongly reject the diagnosis. This is reported in almost half of cases of the syndrome (27). Cytologic features of hemophagocytosis should therefore not be considered the gold standard for the diagnosis of hemophagocytic syndrome.

Variables included in our multivariate analysis were those resulting from a Delphi study involving a panel of experts on the reactive form of hemophagocytic syndrome (20). Among these variables, LDH level was

considered by 75% of the experts to be absolutely required or important, but was not independently associated with the diagnosis of reactive hemophagocytic syndrome according to our analyses. In contrast, other variables that only 52–62% of the experts in the Delphi study considered absolutely required or important, such as low fibrinogen or high triglyceride levels, were strongly associated with a positive diagnosis of hemophagocytic syndrome in our study, confirming that some of the criteria for which no consensus was reached by that group were ultimately useful.

The proposed HScore has several strengths. It is the first available set of criteria developed using appropriate and robust methodology. The selection of variables and the weights assigned to levels of these variables were based on the combined use of a Delphi survey and logistic regression modeling. This differs from previous criteria sets, which were based solely on clinical judgment. Even though the variables we found to be statistically associated with the diagnosis of hemophagocytic syndrome were those generally recognized by others as being helpful in diagnosis (18,23), the design of our study enabled us to estimate the appropriate threshold values for each variable and to assess the appropriate weight of each one in the diagnosis of hemophagocytic syndrome. Second, our large study population consisted of patients with reactive hemophagocytic syndrome of different etiologies. The good performance of the HScore in both the developmental and the validation data sets ensures that the model is correctly calibrated to the range of diseases associated with hemophagocytic syndrome. Finally, data collection required to calculate the HScore is very simple and quick. This ease of use, coupled with the fact that the HScore is freely available online (http://saintantoine.aphp.fr/score/), should result in its widespread acceptance and use in routine practice.

The HScore has some limitations as well. First, it was developed using a retrospective study population, with the possibility of bias in the selection of this population. To minimize this bias and to ensure that no patient fulfilling our inclusion criteria was missed, we selected the study population by reviewing the medical records, the results of bone marrow aspiration, and the ICD-10 classification of patients. We thus verified that the few patients coded as having hemophagocytic syndrome who did not undergo bone marrow aspiration were mostly patients with a recurrence of hemophagocytic syndrome, who therefore could not be included in our analyses. A bias in data recording may also be hypothesized. However, the patients' medical records were reviewed by investigators familiar with the syndrome, using a standardized procedure. Moreover, because of the severity of the syndrome, only few data were missing at the time of bone marrow aspiration.

The second main limitation relates to the classification of the patients. This classification was considered the gold standard and was therefore central in the construction of the HScore. In order to ensure that the cases and the controls were adequately classified, 4 investigators with extensive expertise regarding hemophagocytic syndrome analyzed all of the files independently, and were blinded with regard to the decisions made by the other investigators and the results of the Delphi study run at the same time. Since there is no pathognomonic finding in hemophagocytic syndrome and thus no gold standard for the diagnosis, we believe the global procedure that was used enabled the investigators to classify the patients as correctly as possible. Patients for whom no consensus was reached were classified as having undetermined hemophagocytic syndrome status and were therefore not included in the analyses performed for construction and validation of the HScore. Not surprisingly, the distribution of HScores among these patients was wide, reflecting the broad spectrum of this "difficult to diagnose" group of patients. Therefore, although it is not possible to evaluate the performance of the HScore in this population, this score might be considered the best available tool to date for guiding diagnosis decisions in all patients.

Another limitation is related to the fact that the diagnostic approach to hemophagocytic syndrome may partly depend on the underlying disease, each disease being associated with particular biologic abnormalities (e.g., elevated white blood cell counts and serum ferritin levels in patients with Still's disease, cytopenia in patients with systemic lupus erythematosus). Therefore, the cutoff values for laboratory criteria may depend on the underlying disease, and ideally, patients should have been grouped by underlying disease and these cutoffs assessed separately for each group. However, the rarity of hemophagocytic syndrome makes this difficult.

Finally, in this initial study we chose to focus more on the development of the score than on its validation. Therefore, we decided to include only 10% of the overall population with a positive or negative hemophagocytic syndrome classification (n = 27) in the validation data set. Although the score performances found in this validation cohort make us confident about the robustness of HScore, prospective validation of the score in other samples of patients is needed before it is recommended for widespread use, given the small num-

ber of patients used to cross-validate the instrument combined with the retrospective design of the study.

In conclusion, we have described the construction of a scoring system that is designed to help clinicians in diagnosing hemophagocytic syndrome in routine practice. The score has been made freely available online in order to facilitate its use. Further research should evaluate the robustness of the HScore in other series of patients and should assess its performance in the diagnosis of the primary form of hemophagocytic syndrome.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Fardet had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Fardet, Galicier, Lambotte, Coppo, Hejblum.

Acquisition of data. Fardet, Galicier, Lambotte, Coppo.

Analysis and interpretation of data. Fardet, Galicier, Lambotte, Marzac, Aumont, Chahwan, Coppo, Hejblum.

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