Predicting macrophage activation syndrome (MAS) in childhood-onset systemic lupus erythematosus (cSLE) patients at diagnosis

Maya Gerstein[1, 2], R. Ezequiel Borgia[1,3], Daniela Dominguez[1], Brian M Feldman[1], Fangming Liao[1], Deborah Levy[1], Lawrence Ng[1], Mohamed Abdelhaleem[4], Earl D Silverman[1], Linda T Hiraki[1]

1: Division of Rheumatology, The Hospital for Sick Children, Toronto, Canada
2: Pediatric Rheumatology unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer
3: Department of Pediatrics, College of Medicine, University of Florida, Gainesville, Florida, USA
4: Division of Haematopathology, The Hospital for Sick Children, Toronto, Canada

Correspondence:
Linda T. Hiraki, MD, FRCPC, ScD
Clinician Scientist, Division of Rheumatology
Scientist, Research Institute
The Hospital for Sick Children
PGCRL, 686 Bay Street | Toronto ON M5G 0A4
Email: linda.hiraki@sickkids.ca

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ABSTRACT:

Purpose: Macrophage activation syndrome (MAS), a life-threatening inflammatory complication, is increasingly recognized in childhood-onset SLE (cSLE). It can be a challenge to differentiate active cSLE from MAS. We generated decision rules for discriminating MAS from active cSLE in newly diagnosed patients.

Methods: We conducted a retrospective cohort study of consecutive, newly diagnosed, active cSLE patients with fever, requiring hospital admission to SickKids from January 2003 - December 2007 (cohort 1), and January 2008 - December 2013 (Cohort 2). All patients met ≥4 ACR or SLICC criteria, were steroid naïve and infection free. MAS was diagnosed based on expert opinion. Recursive partitioning was applied to each cohort to derive a decision rule based on clinical and laboratory features, distinguishing MAS from non-MAS cSLE. Each decision rule was applied to the alternate, independent cohort. Sensitivity and specificity of these decision rules were compared to existing criteria.

Results: Cohort 1 (n=34) and cohort 2 (n=41) each had 10 MAS patients. Recursive partitioning in cohort 1 identified ferritin ≥699 µg/L, as the sole best discriminator between MAS and non-MAS patients (R^2=0.48) and in cohort 2 ferritin ≥1107 µg/L, followed by lymphocytes < 0.72 $x10^3/mm^3$ were the best discriminators for MAS (R^2=0.52). Cross-validation of our decision rules maintained 90-100% sensitivity and 65-85% specificity.

Conclusions: Our decision rule demonstrated improved performance compared to preliminary guidelines for MAS in cSLE from the Lupus Working Group of the Paediatric Rheumatology European Society, and familial Hemophagocytic Lymphohistiocytosis diagnostic criteria. Validation in independent cohorts is required.

Word count: 246 (limit 250)

INTRODUCTION

Macrophage activation syndrome (MAS) is a potentially life-threatening complication of inflammatory disorders including childhood-onset systemic lupus erythematosus (cSLE) and other pediatric and adult rheumatic diseases.(1-4) MAS, a secondary form of familial hemophagocytic histiocytosis (fHLH), is so named for the marked clinical and laboratory similarity of the diseases. Both MAS and fHLH are characterized by excessive activation of differentiated macrophages with the resultant presence of hemophagocytic macrophages in the bone marrow, liver, spleen and/or lymph nodes. These activated macrophages phagocytose multiple hematopoietic lineages, contributing to pancytopenia, exacerbated by systemic inflammatory responses.(5, 6) Other features include coagulopathy, hypertriglyceridemia, hypofibrinogenemia and hyperferritinemia.(7) The clinical presentation manifests with persistent fever, hepatosplenomegaly, lymphadenopathy and central nervous system dysfunction.(8)

The clinical features of active SLE include many of the clinical features of MAS and, in particular, patients with SLE can present with hepatosplenomegaly, lymphadenopathy and central nervous system (CNS) dysfunction. Similarly, the characteristic laboratory features of MAS including pancytopenia, coagulopathy, hypertriglyceridemia, and hyperferritinemia are seen in active SLE.(9-11) Due to the overlapping features, it can be difficult to differentiate active SLE from MAS.(2, 12-14) In 2009, the Lupus Working Group of the Paediatric Rheumatology European Society (PReS) developed preliminary guidelines for the diagnosis of MAS in cSLE, but these have not been validated.(3)

The purpose of this study was to develop a decision rule to differentiate MAS from active cSLE among newly diagnosed, treatment naïve cSLE patients. Our second aim was to compare the performance of our decision rules (sensitivity and specificity) with those from the PReS

Lupus Working Group preliminary criteria for MAS in cSLE and the 2004 fHLH diagnostic criteria.(3, 15)

PATIENTS AND METHODS

Patient population:

We restricted our study population from our Lupus Clinic database to include patients admitted to The Hospital for Sick Children, Toronto, for newly diagnosed cSLE between January 2003 to December 2007 (Cohort 1), and January 2008 to December 2013 (Cohort 2). We extracted prospectively collected clinical and laboratory data. All patients met ≥4 ACR and/or SLICC classification criteria for SLE.(16, 17) We reviewed all hospital admissions lasting a minimum of 3 days, and occurring within 2 months prior to, and up to 12 months following cSLE diagnosis. From among the admitted patients we identified those with a clinical diagnosis of MAS by the treating pediatric rheumatologist during the admission (expert opinion). Patients were excluded for: 1) absence of fever; 2) prior steroid use at time of presentation and diagnosis; 3) isolated infection; or 4) elective admission for treatment or procedures. Institutional Research Ethics Board (REB) approval was obtained prior to initiation of the study (REB #1000035186).

Clinical and laboratory variables:

We reviewed the clinical and laboratory features of all patients included in our cohort, during hospital admission. Clinical features of MAS included documentation of fever (38.5°C or higher), CNS dysfunction (irritability, seizures, severe headache, hallucinations, disorientation or coma), splenomegaly, hepatomegaly and hemorrhagic manifestations (purpura, easy bruising or

mucosal bleeding). Laboratory parameters included: complete blood count and differential (hemoglobin, white blood cell (WBC), neutrophil, lymphocyte and platelet counts), direct Coombs/direct antiglobulin test (DAT), triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), albumin, ferritin, fibrinogen, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, international normalized ratio (INR), activated partial thrombin time (APTT), D-dimer, C3, C4, IgG, urea, serum creatinine, erythrocyte sedimentation rate (ESR), sodium, calcium, C-reactive protein (CRP). The prevalence of SLE features was compared between MAS and non-MAS patients within each cohort using a Fisher's exact test and a significance threshold adjusted for multiple comparisons ($P< 0.003$).

When available, bone marrow aspirates/biopsies were reviewed for evidence of hemophagocytosis. Additional MAS markers, soluble CD25 (sCD25/soluble IL-2 receptor alpha chain), CD163, and NK cell activity were measured in 4 patients. Since bone marrow aspirates/biopsies and these additional MAS markers were not tested in all participants, they were excluded factors in recursive partitioning. When testing the performance of the fHLH criteria in our population, we restricted to patients with bone marrow aspirates/biopsies and did not include features of sCD25 and NK cell activity.

Statistical analysis

Within each cohort (cohort 1: 2003 to 2007, cohort 2: 2008 to 2013), patients were assigned to one of two mutually exclusive groups: a) MAS and b) non-MAS. We performed recursive partitioning in each cohort using baseline quantitative laboratory measures and binary clinical features to deriving a decision rule for identifying MAS (RStudio 0.99.902.(18)). To maximize

information and avoid overfitting we compared parameters of fit (area under the receiver operating characteristic (ROC) and R^2) with complexity and cross-validation error. We tested the performance of each decision rule by applying the rule to the alternate, independent cohort (i.e. cohort 1 derived decision rule was applied to cohort 2 and vice-versa). We calculated the sensitivity and specificity of the decision rule for identifying MAS in each cohort. Lastly, we completed recursive partitioning on the total patient population to increase the sample size and power of our analysis. The sensitivity and specificity of the existing primary fHLH and the PReS Lupus Working Group preliminary criteria for MAS in cSLE were determined as applied to our study cohort. In sensitivity analyses, we added the ratio of baseline serum ferritin (ng/mL) to erythrocyte sedimentation rate (mm/hr) to the baseline laboratory and clinical parameters and re-generated the decision rules.

RESULTS

We reviewed 406 newly diagnosed cSLE patient charts, of which 214 patients had at least one hospital admission. After excluding 138 patients based on our exclusion criteria, our study cohort included 34 patients in cohort 1 (10 with MAS and 24 without MAS) and 41 in cohort (10 with MAS and 31 without MAS). In 99% of patients, hospital admission preceded or coincided with SLE diagnosis, with only one patient requiring admission 28 days after diagnosis. All patients were naïve to corticosteroid treatment prior to presentation.

The mean age at hospital admission was 14.0 years (SD 2.5 years) in cohort 1 and 13.6 years (SD 2.5 years) in cohort 2 ($P = 0.52$). The majority of patients were female (cohort 1: 85%; cohort 2: 81%, $P = 0.76$) (Table 1). The prevalence of specific SLE manifestations were comparable in cohorts 1 and 2 (Table 1).

Regarding MAS manifestations, none of the clinical features typically associated with MAS, was found to be significantly different between the MAS and non-MAS groups (Table 2). Bone marrow (BM) aspirate and/or biopsies were performed on 43 patients (20 in cohort 1, and 23 in cohort 2). In cohort 1, significant hemophagocytosis was present 3/6 MAS patients, and 3/14 non-MAS patients who had BM aspiration and biopsy (50% vs. 21%, p-value = 0.30). In cohort 2, hemophagocytosis was evident on 2 of the 9 MAS patient BM specimens and 1 of the 14 non-MAS specimens (22% vs. 7%, p-value = 0.54).

Recursive partitioning

We used recursive partitioning to determine cut-off values for laboratory tests differentiating patients with MAS from patients without MAS in each cohort (Figure 1). At the outset the pretest probability of a patient having MAS was 27% (that is 20/75 of our total cohort had MAS). In Cohort 1, recursive threshold testing identified ferritin as the sole parameter differentiating MAS vs. non-MAS patients, with a cut-off value of ≥699 µg/L (R^2 =0.48). Testing this decision rule in cohort 2, demonstrated a sensitivity of 90% and a specificity of 81% for MAS (area under the receiver operating characteristic (AUC) = 0.87, Supplemental Figure 1a).

When we performed recursive partitioning in cohort 2 (Figure 2 including hierarchy of testing), we derived a decision rule that best differentiated MAS from non-MAS patients, identified the following thresholds: 1) Ferritin ≥ 1107 µg/L; and 2) Lymphocytes <$0.72x10^3$ /mm^3, with a R^2= 0.52. When we tested this decision rule in cohort 1, the sensitivity was 90% and specificity of 63% (AUC = 0.77, Supplemental Figure 1b).

We next combined the 2 cohorts into a single cohort and generated a 3rd decision rule for differentiating patients with MAS from patients without MAS (Figure 3 including hierarchy of testing). Serial cut-off testing resulted in the following algorithm: 1) Ferritin ≥ 669 μg/L; 2) WBC <2.25x10^3 /mm^3, which resulted in R^2= 0.62.

In testing the performance of fHLH criteria restricted to patients with bone marrow aspirates/biopsies, we observed that in cohort 1, 3/6 patients with MAS, and 2/14 without MAS met > 5 criteria for fHLH(15) (Table 3, Supplemental Table 1). This resulted in a sensitivity of 50%, and specificity of 86%. In cohort 2, 4/9 patients with MAS and none of the non-MAS met > 5 criteria for fHLH. This resulted in a sensitivity of 44%, and specificity of 100%. In the combined cohort 1 and 2, 7/15 patients with MAS and 2/28 of the non-MAS patients met fHLH criteria, which resulted in a sensitivity of 47% and a specificity of 93%. Testing the PReS Lupus Working Group preliminary criteria for MAS in cSLE in cohort 1 demonstrated a sensitivity of 100% and specificity of 17%, and in cohort 2 a sensitivity of 100% and specificity of 32%. In the combined cohort, all 20 patients with MAS and 41/55 non-MAS met the PReS Lupus Working Group preliminary criteria for MAS in cSLE resulting in a sensitivity of 100% and a specificity of 25%.

Sensitivity analyses adding the baseline serum ferritin/ESR ratio did not change the resultant decision rule for cohorts 1 or 2, nor in the combined cohort.

DISCUSSION

MAS is a life-threatening complication of SLE that is difficult to differentiate from active SLE without MAS, due to overlapping clinical and laboratory manifestations.(4, 13, 19) We developed criteria distinguishing MAS in cSLE patients, from those with active SLE alone, using recursive partitioning, in two independent cohorts of treatment naïve patients with cSLE. Our

new criteria demonstrated improved discriminatory power compared to existing diagnostic criteria for fHLH(15) and the PReS Lupus Working Group preliminary criteria for MAS in cSLE.(3)

We found the fHLH diagnostic criteria had poor sensitivity for diagnosing MAS secondary to SLE, likely due to limited availability of special tests such as NK cell activity, or the extreme thresholds for laboratory abnormalities such as cytopenias and ferritin levels. Another limitation of applying the existing fHLH criteria to cSLE patients, is the central role of bone marrow biopsy for fHLH diagnosis.(15) Bone marrow aspirate or biopsy offers limited utility for MAS diagnosis in rheumatic diseases. As such, preliminary guidelines for MAS in cSLE, do not require bone marrow examination.(3, 8) Consistent with observations in fHLH,(20) and other autoimmune diseases,(1, 4) we demonstrated that the presence of hemophagocytosis on bone marrow aspirate/biopsy is neither sensitive nor specific for the diagnosis of secondary MAS in cSLE patients.(20) Requiring a BM specimen for MAS diagnosis will likely delay diagnosis and increase the mortality associated with MAS.

Recognizing the limited sensitivity of fHLH diagnostic criteria for identifying MAS in cSLE patients, the PReS Lupus Working Group proposed guidelines for the diagnosis of MAS in cSLE that include many features patients with SLE manifest at the time of diagnosis.(3) Although these PReS guidelines had improved sensitivity compared to the fHLH diagnostic criteria for MAS in SLE, they were also less specific than fHLH criteria when applied to our inpatient cohorts, since fever,(10, 21) splenomegaly,(10) cytopenias ($\geq$ 2/3 lineages),(10) hypertriglyceridemia,(22) hemophagocytosis in bone marrow,(20) low NK cell activity,(23) elevated ferritin,(11) and elevated sCD25 levels,(24, 25) are frequently seen in patients with active SLE.

Since concern for MAS often arises in hospitalized SLE patients, we aimed to develop MAS criteria with improved power to discriminate MAS from active SLE, over existing fHLH and PReS Lupus Working Group criteria. We restricted our cohort to patients admitted to hospital with documented fever, and no prior exposure to corticosteroids. This selection strategy not only ensured that our MAS case and non-MAS control populations represented real-life clinical scenarios, but that laboratory parameters were unaffected by past medication exposures. When we applied the PReS Lupus Working Group criteria for MAS in SLE, to our cohort we found that 100% of the cSLE with MAS patients met the criteria. However, there was a high false positive rate as well, with 75% of the newly diagnosed cSLE patients without the clinical diagnosis of MAS also meeting the criteria.

We created 3 decision rules for MAS in cSLE, using recursive partitioning, one rule from each cohort, and one derived from both cohorts together. Recursive partitioning used the cohorts data to create the best fitting model, which in this case demonstrated an R^2 value of 0.48, 0.52, 0.62 (in cohorts 1,2 and both respectively). However, there was a risk of overfitting a model and generating an algorithm specific to the dataset from which it arose. Hence, the true test of the model's performance is in its application to an independent cohort. Our study was designed specifically to overcome this obstacle; having 2 cohorts derived from the same population, being managed by the same experts and laboratories; therefore, we were able to apply each cohort's rule on the independent parallel cohort, using it as a testing cohort.

Our proposed criteria for MAS in cSLE are similar to those proposed by the PReS Lupus Working Group. All 3 rules identified ferritin as the first parameter distinguishing between MAS and non-MAS among newly diagnosed, treatment naive cSLE patients. However, our threshold for hyperferritinemia was higher than the one proposed by the PReS Lupus Working Group (≥

699 µg/L compared with >500µg/L). As with the PReS criteria, we also found that cytopenia was informative, specifically lymphopenia (cohort 2 decision rule) and leukopenia (Total cohort decision rule) as the second most informative discriminators between MAS and non-MAS. A prior study demonstrated improved sensitivity and specificity of serum ferritin/ESR ratio for diagnosing MAS in sJIA populations, over ferritin alone.(26) Our sensitivity analyses added the baseline ferritin/ESR ratio to our recursive partitioning models. However, we did not observe improved discriminatory power for MAS in cSLE, of the ferritin/ESR ratio over ferritin and cytopenias.

Our proposed MAS criteria in cSLE, identified the same informative laboratory parameters as the PReS Lupus Working Group. However the more extreme thresholds we propose, improved the specificity of our criteria over those proposed by PReS Lupus Working Group. In clinical application, it may be appropriate to consider a sequential application of criteria, beginning with the most sensitive criteria from the Lupus Working Group of PReS to ensure complete identification of all MAS cases, followed by more specific criteria proposed by our work, to reduce the number of false positive MAS cases.

Our study findings should be considered in light of some potential limitations. There is no diagnostic gold-standard for MAS, which necessitated the reliance on pediatric rheumatologist diagnosis. However, each MAS diagnosis was independently verified by one investigator, who reviewed the entire disease course in hospital including therapy response. Any disputed diagnoses were discussed and validated by all the investigators.(27) Also, we did not have access to a third independent cohort in which to test our final criteria's performance. Therefore we concluded that these proposed criteria should be validated.

Our study had a number of strengths. We were able to focus on a large number of steroid-naïve, acutely ill cSLE patients at disease presentation, in two independent cohorts separated by era. In this way our study cohorts represented an ill cSLE population, in whom MAS diagnostic criteria would have the greatest clinical benefit. Making an early and timely diagnosis of MAS is critical since the therapy differs for MAS and active SLE disease. Our study also demonstrated an increased prevalence of MAS (26%) among our selected cohort of cSLE patients requiring hospitalization. This is compared to our prior work that reported an MAS prevalence of 9% in our expanded cSLE population.(27) This higher MAS prevalence in hospitalized cSLE patients, emphasizes the importance of considering MAS as a complicating disease process in SLE patients requiring hospitalization, rather than attributing illness to SLE disease activity alone.

Our proposed criteria for diagnosing MAS in patients with cSLE, identified ferritin, lymphocyte and leukocyte counts as the most informative factors in discriminating MAS from active SLE. Our criteria have demonstrated improved sensitivity and specificity for MAS in our cohorts, when compared to current diagnostic criteria for fHLH and the PReS Lupus Working Group preliminary guidelines for MAS in cSLE. Testing our criteria in independent cSLE and adult-onset SLE cohorts, would provide additional value for the generalizability and utility of our proposed criteria.

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Figure Legends

Figure 1. Recursive partitioning decision rule derived from Cohort 1
The boxes summarize the numbers of MAS and non-MAS patients in Cohort 1, and the decision node of ferritin threshold 699 ug/mL, terminal branches and respective sample sizes. The R2 of 0.69 reflects the decision rule fit in Cohort 1.

Figure 2. Recursive partitioning decision rule derived from Cohort 2
The boxes summarize the numbers of MAS and non-MAS patients in Cohort 2, and the decision node of ferritin threshold 1107 ug/mL, followed by Lymphocyte threshold count of 0.72 x $10^3/mm^3$, terminal branches and respective sample sizes. The R2 of 0.52 reflects the decision rule fit in Cohort 2.

Figure 3. Recursive partitioning decision rule derived for both cohorts
The boxes summarize the numbers of MAS and non-MAS patients in the total cohort, and the decision node of ferritin threshold 699 ug/mL, followed by white blood cell (WBC) threshold count of 2.3 x $10^3/mm^3$, terminal branches and respective sample sizes. The R2 of 0.62 reflects the decision rule fit in the total cohort.

Table 1: Systemic Lupus Erythematosus Features at diagnosis

	Cohort 1 (n=34)		Cohort 2 (n=41)	
Characteristics	**MAS (n=10) (%)**	**non-MAS (n=24) (%)**	**MAS (n=10) (%)**	**non-MAS (n=31) (%)**
Mean age at diagnosis (range) years	14.9 ± 1.5 (11.9-16.5)	13.6 ± 2.8 (9-17.1)	13.6 ± 2.9 (7.8-16.6)	13.6 ± 2.4 (8.8-17.2)
Female	8 (80)	21 (88)	8 (80)	25 (81)
Organ System Involvement				
Arthritis	5 (50)	19 (79)	8 (80)	18 (58)
Mucocutaneous involvement:				
Malar rash	8 (80)	8 (33)	5 (50)	24 (77)
Other rash	5 (50)	11 (46)	3 (30)	7 (22)
Oral ulcers	5 (50)	8 (33)	3 (30)	8 (25)
Alopecia	3 (30)	14 (58)	2 (20)	6 (19)
Photosensitivity	2 (20)	6 (25)	2 (20)	5 (16)
Nasal ulcers	1 (10)	4 (17)	2 (20)	3 (9)
Digital ulcers	0	1 (4)	2 (20)	2 (6)
Lupus Nephritis (LN) (any):	3 (30)	11 (46)	5 (50)	14 (44)
Mesangial (Class II)	0	1 (4)	2 (20)	1 (3)
Focal Proliferative (Class III)	1 (10)	4 (17)	2 (20)	5 (16)
Diffuse Proliferative (Class IV)	1 (10)	4 (17)	1 (10)	6 (19)
Membranous (Class V)	1 (10)	4 (17)	0	3 (9)
Nephrotic Syndrome	1 (10)	5 (21)	0	5 (16)
Central nervous system (CNS) (any):	2 (20)	10 (42)	1 (10)	7 (23)
Psychosis	0	3 (13)	1 (10)	1 (3)
Cerebrovascular Disease	2 (20)	5 (21)	0	1(3)
Cognitive dysfunction	0	6 (25)	1 (10)	2 (6)
Pericarditis	0	6 (25)	1 (10)	9 (28)
Pleuritis	1 (10)	5 (21)	3 (30)	9 (29)
Myositis	0	2 (8)	0	2 (6)
Diffuse lymphadenopathy	6 (60)	8 (33)	2 (20)	7 (22)
Raynaud's	1 (10)	2 (8)	0	2 (6)
Fatigue	9 (90)	17 (71)	9 (90)	17 (53)
Fever	10 (100)	24 (100)	10 (10)	32 (100)
Weight loss	4 (40)	14 (58)	4 (4)	16 (50)
Anorexia	5 (50)	9 (38)	6 (6)	11 (34)
Headache	3 (30)	6 (25)	1 (10)	6 (19)
Autoantibodies:				
ANA	10 (100)	24 (100)	10 (100)	31 (100)
Anti-dsDNA	8 (80)	21 (88)	9 (90)	25 (81)
Anti-Sm	6 (60)	11 (46)	4 (40)	13 (41)
Anti-RNP	7 (70)	12 (50)	4 (40)	28 (90)
Anti-Ro	4 (40)	12 (50)	5 (50)	15 (47)
Anti-La	1 (10)	3 (13)	2 (20)	4 (13)
Antiphospholipid (Any):	3 (30)	19 (79)	5 (50)	9 (28)
Anti-cardiolipin	3 (30)	19 (79)	4 (40)	7 (22)
Lupus anticoagulant	1 (10)	3 (13)	1 (10)	4 (13)
Rheumatoid Factor	0	2 (8)	0	0
Hematologic:				

Thrombocytopenia	6 (60)	7 (29)	7 (70)	7 (23)
Lymphopenia	9 (90)	15 (63)	9 (90)	21 (68)
Coombs' positive hemolytic anemia	2 (20)	10 (42)	7 (70)	16 (52)
Positive DAT	7 (70)	18 (75)	8 (80)	19 (59)
Leukopenia	1 (10)	0	2 (20)	7 (23)
Neutropenia	0	0	1 (10)	1 (3)

Table 2: HLH clinical features and laboratory results in MAS and non-MAS patients by cohort.

	Cohort 1 (n=34)		Cohort 2 (n=41)	
Clinical Features [n (%)]	**MAS n=10**	**Non-MAS n=24**	**MAS n=10**	**Non-MAS n=31**
Lymphadenopathy	7 (70)	15 (63)	5 (50)	15 (47)
Hepatomegaly	2 (20)	4 (17)	2 (20)	5 (16)
Splenomegaly	1 (10)	7 (29)	5 (50)	7 (22)
Central Nervous System	4 (40)	6 (25)	3 (30)	12 (38)
Bleeding	1 (10)	7 (29)	3 (30)	7 (22)
Hemophagocytosis on BM*	3/6 (50)	3/14 (21)	2/9 (22)	1/14 (7)
Laboratory findings [mean ± SD]				
Hemoglobin (g/L)	97.3 ± 18.6	87.8 ± 14.3	96.4 ± 18.5	94.2 ± 16.7
WBC (x10^9/L)	2.6 ± 1.7	5.1 ± 3.0	2.2 ± 1.3	4.4 ± 2.6
Neutrophils (x10^9/L)	1.31 ± 0.96	2.89 ± 2.37	1.10 ± 0.64	2.65 ± 1.68
Lymphocytes (x10^9/L)	0.62 ± 0.34	1.29 ± 0.67	0.59 ± 0.40	1.22 ± 0.69
Platelet count (x10^9/L)	158 ± 127	192 ± 176	115 ± 54	210 ± 144
AST (U/L)	163 ± 171	67 ± 56 †	190 ± 204	78 ± 119
ALT (U/L)	78 ± 69	37 ± 24 †	77 ± 57	69 ± 155
LDH (U/L)	2094 ± 1348	1055 ± 443(n=18)	2046 ± 1019 †	1167 ± 1157 ‡
Albumin (g/L)	27 ± 9	31 ± 8 ‡	28 ± 7 †	28 ± 7 †
Ferritin (ug/L)	7579 ± 16,647 †	757 ± 1282 (n=16)	2796 ± 2164 †	808 ± 1591 (n=25)
Fibrinogen (g/L)	3.3 ± 1.4	4.3 ± 1.6 (n=11)	2.7 ± 0.7	4.1 ± 1.5 (n=22)
D.Dimer (ug/mL)	4.2 ± 10.1	1.26 ± 0.96	4.8 ± 2.8	3.2 ± 4.1(n=23)
Triglycerides (mmol/L)	2.2 ± 1.5	2.4 ± 1.34 (n=20)	3.2 ± 1.23 †	2.3 ± 1.9 (n=27)
INR	1.1 ± 0.2	1.0 ± 0.4 †	1.1 ± 0.1	1.0 ± 0.1 †
APTT (sec)	37 ± 8	31 ± 4 †	35 ± 7	32 ± 7 †
C3 (g/L)	0.43 ± 0.33	0.59 ± 0.49	0.49 ± 0.48	0.62 ± 0.33
C4 (g/L)	0.086 ± 0.084	0.095 ± 0.085	0.082 ± 0.116	0.100 ± 0.085
Urea (mmol/L)	7.0 ± 5.6	5.6 ± 3.17	3.71 ± 1.73	5.28 ± 3.89 †
Creatinine (umol/L)	88 ± 53	69 ± 33	67 ± 22	66 ± 41
Calcium (mmol/L)	1.90 ± 0.37	2.04 ± 0.16	1.96 ± 0.21	2.05 ± 0.29
Sodium (mmol/L)	139 ± 5	136 ± 5	136 ± 5 †	139 ± 3 ‡
ESR (mm/Hr)	81 ± 43	102 ± 22 ‡	70 ± 52	100 ± 37
CRP (mg/L)	21.8 ± 39.7 (n=8)	27.0 ± 48.4 ‡	30.6 ± 34.3	25.4 ± 40.4 †
IgG (g/L)	19.5 ± 8.1 (n=7)	20.2 ± 5.9 (n=21)	18.3 ± 4.2	22.3 ± 10.3 †

*BM available in a subset of patients. Number reported in each group.

† Laboratory data missing on 1 subject.

‡Laboratory data missing on 2 subjects.

Table 3: Performance of existing criteria for HLH and MAS diagnosis

	Cohort 1 (n=34)		Cohort 2 (n=41)		All (n=76)	
Criteria	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Cohort 1 decision rule	-	-	90	85	-	-
Cohort 2 decision rule	90	63	-	-	-	-
PReS Lupus Working Group proposed criteria for MAS in cSLE	100	17	100	32	100	25
Familial Hemophagocytic Lymphohistiocytosis criteria*	50	86	44	100	47	93

- Estimation of sensitivity and specificity in these cohorts would be overfit since these cohorts gave rise to the decision rule.

*Restricted to patients with bone marrow aspirates/biopsies (Cohort 1: n=20; Cohort 2: n=23). sILR and NK cell activity were not included in sensitivity and specificity calculations.

Figure 1: Recursive partitioning decision rule derived from Cohort 1

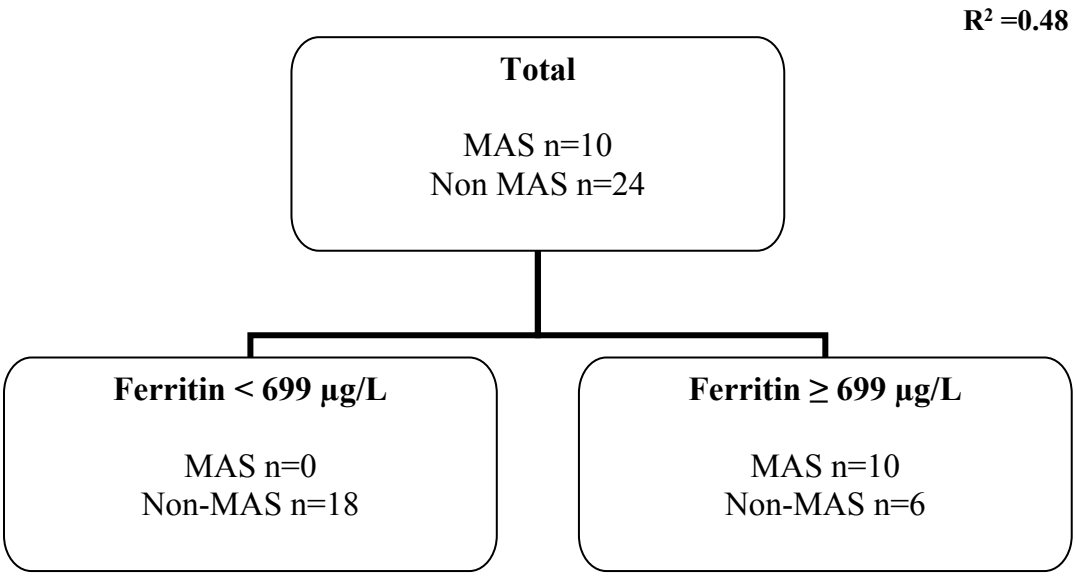

Figure 2: Recursive partitioning decision rule derived from Cohort 2

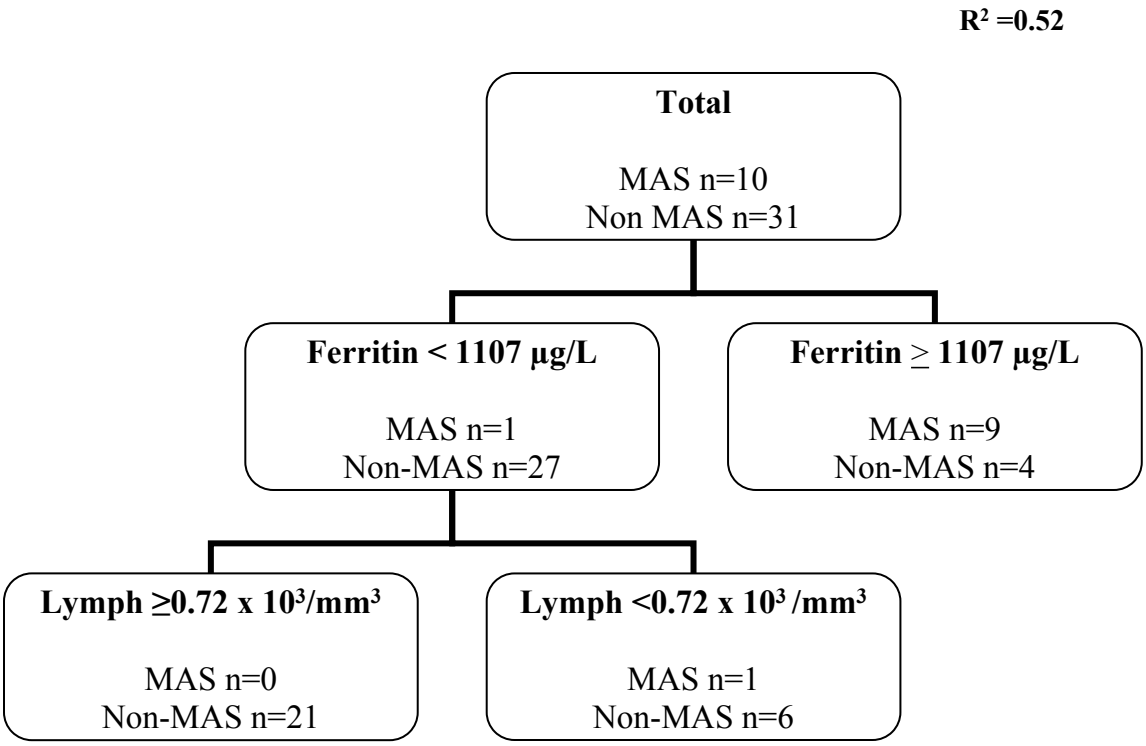

Figure 3: Recursive partitioning decision rule derived for both cohorts