

**Original Article****Soluble ST2 and CD163 as Potential Biomarkers to Differentiate Primary Hemophagocytic Lymphohistiocytosis from Macrophage Activation Syndrome**Zhuo Gao¹, Yini Wang¹, Jingshi Wang¹, Jia Zhang¹ and Zhao Wang¹.¹ Department of Hematology, Beijing Friendship Hospital, Capital Medical University, Beijing, China.**Competing interests:** The authors have declared that no competing interests exist.

Abstract. The differentiation of primary hemophagocytic lymphohistiocytosis (pHLH) and macrophage activation syndrome (MAS) poses a challenge to hematologists. The aim of this study was (1) to compare the levels of soluble ST2 (sST2), sCD163, IFN- γ , IL-10, IL-18, TNF- α and Serum soluble interleukin-2 receptor (sCD25) in patients with pHLH and MAS and (2) to investigate whether they can help differentiate the two diseases. A total of 52 participants were recruited in this study, including 12 pHLH patients, 20 MAS patients, and 20 healthy subjects. We measured the levels of sST2, sCD163 and sCD25 in serum by ELISA. The serum levels of IFN- γ , IL-10, IL-18, and TNF- α were detected using a Luminex 200 instrument. The serum levels of sST2 and sCD163 in MAS patients were markedly higher than that in pHLH patients (363.13 ± 307.24 ng/ml vs 80.75 ± 87.04 ng/ml, $P = 0.004$; 3532.72 ± 2479.68 ng/ml vs 1731.96 ± 1262.07 ng/ml, $P = 0.046$). There was no significant difference in the expression of IFN- γ (306.89 ± 281.60 pg/ml vs 562.43 ± 399.86 pg/ml), IL-10 (20.40 ± 30.49 pg/ml vs 8.3 ± 13.14 pg/ml), IL-18 (463.33 ± 597.04 pg/ml vs 1247.82 ± 1318.58 pg/ml), TNF- α (61.48 ± 84.69 pg/ml vs 106.10 ± 77.21 pg/ml), and sCD25 (21062.1 ± 18515.26 pg/ml vs 11074.78 ± 11149.96 pg/ml) between pHLH and MAS. Patients with pHLH and MAS show some differences in cytokine profiles. The elevated levels of IFN- γ , IL-10, TNF- α , IL-18, and sCD25 can contribute to the diagnosis of HLH, but may not discriminate pHLH from MAS. Levels of sST2 and sCD163 may serve as markers to distinguish pHLH from MAS.

Keywords: Hemophagocytic lymphohistiocytosis; Macrophage activation syndrome; sST2; sCD163; Biomarkers.**Citation:** Gao Z., Wang Y., Wang J., Zhang J., Wang Z. Soluble ST2 and CD163 as potential biomarkers to differentiate primary hemophagocytic lymphohistiocytosis from macrophage activation syndrome. *Mediterr J Hematol Infect Dis* 2019, 11(1): e2019008, DOI: <http://dx.doi.org/10.4084/MJHID.2019.008>**Published:** January 1, 2019**Received:** August 8, 2018**Accepted:** November 26, 2018This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by-nc/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.Correspondence to: Zhao Wang, Department of Hematology, Beijing Friendship Hospital, Capital Medical University, 95 Yong An Road, Xicheng District, Beijing 100050, China. Tel/Fax: +86-10-63138303. E-mail: wangzhao@ccmu.edu.cn

Introduction. Hemophagocytic lymphohistiocytosis (HLH) is a clinical syndrome manifested by fever, hepatosplenomegaly, cytopenia, and hemophagocytosis in bone marrow, liver, spleen or lymph nodes. HLH is classified into primary HLH (pHLH) and secondary HLH (sHLH). The pHLH is triggered by genetic mutations whereas sHLH is mainly associated with infection, cancer or autoimmune diseases.¹ According to the diagnostic guidelines HLH-2004,² the diagnosis for pHLH requires definite evidence of a genetic defect.

Nevertheless, genetic sequencing is time-consuming and may unnecessarily delay the administration of specific therapy. Several indicators such as natural killer (NK) cell function, perforin (PRF1), granzyme, SLAM-Associated protein (SAP), an X-linked inhibitor of apoptosis protein (XIAP), MUNC13-4, Syntaxin11, LYST, and ITK protein levels may provide a quick prediction of pHLH. However, the tests for those indicators are difficult to popularize in a short time. Thus the identification of pHLH is quite difficult, if not

impossible, to achieve. MAS is a subtype of sHLH with an approximate mortality rate of 8-22%.³⁻⁵ It is a severe complication of rheumatic diseases caused by excessive activation and expansion of T lymphocytes and macrophages that exhibit hemophagocytic activity.⁶ MAS is a serious, potentially fatal state associated with many rheumatologic diseases including systemic juvenile idiopathic arthritis (SJIA), adult-onset still disease (AOSD), rheumatoid arthritis, systemic lupus erythematosus (SLE), Kawasaki disease (KD), and dermatomyositis.⁷⁻¹² It is clinically characterized by fever, hepatosplenomegaly, lymphadenectasis, severe anemia, liver dysfunction, disseminated intravascular coagulation (DIC) and central nervous system involvement.¹³

HLH, regardless of its subtypes, has an aggressive clinical course with a high mortality rate. In spite of recent advances in the treatment of HLH, therapeutic regimens are often empirically based, and patients' responses to therapies differ sharply. Better prognosis relies heavily on early diagnosis, timely and comprehensive treatments. Therapy for HLH should center on the suppression of the hyperinflammatory state and treatment of any existing HLH triggers.^{14,15} In pHLH, hematopoietic stem cell transplantation (HSCT) will be eventually needed for a full recovery, whereas a pulse of high-dose corticosteroids with or without cyclosporine A (CsA) will work in most MAS patients.¹⁵ Thus, the differentiation of the two subtypes is essential not just for the assessment of a patient's condition but also for the subsequent selection of appropriate treatment. Unfortunately, apart from the similar clinical manifestations with pHLH patients, patients with MAS may also present decreased NK cell activity, lower perforin expression, and single-nucleotide polymorphisms of *UNC13D* and *PRF1* genes.¹⁶ Therefore, it poses a great challenge for us to make a distinction between them with the absence of genetic testing.

In recent years, the role of cytokines in HLH has gained extensive attention and studies on cytokines may open up new avenues in the diagnosis and differential diagnosis of HLH. Increasing evidence suggests that IL-10, IFN- γ , IL-18, TNF- α , and sCD25 play an important role in the pathogenesis of HLH.¹⁷⁻²¹ Besides, Rood²² indicates that disruption of ST2 signaling in the murine model of Family HLH (FHL) can influence the immune regulation and the blockade of ST2 may be a novel therapeutic strategy for FHL. In addition, CD163 has been shown to serve as a potential biomarker for HLH and relevant diseases.²³ Unfortunately, few studies have been focused on the expression of these cytokines in the subtypes of HLH.

In this study, we measured the expression levels of sST2, sCD163, IL-10, IFN- γ , IL-18, TNF- α , and sCD25 and analyzed their role as possible markers to distinguish pHLH from MAS.

Patients and Methods.

Patients. Diagnosis of HLH was based on the criteria set in the HLH-2004.² MAS patients were identified according to the international consensus published in 2011,²⁴ and all the MAS patients meet the HLH-2004 criterion. Patients were confirmed as pHLH based on the evidence of a documented molecular mutation. The evaluation of treatment efficacy was described in our previous study.²⁵ A total of 32 patients was recruited in this study, including 12 pHLH patients, 20 AOSD associated MAS patients presented at the Department of Hematology at Beijing Friendship Hospital from January 2015 to March 2018. Additionally, 20 healthy subjects were invited to participate as controls. This study was approved by the ethics committee of Beijing Friendship Hospital, and informed consent forms were signed by all of the subjects prior to participation in this study. All experiments were performed in accordance with the approved guidelines and regulations.

Sample collection. Peripheral venous blood was collected in the serum-separating tube from patients with pHLH, MAS and healthy controls. The blood was centrifuged at 3000 rpm for 10 min, and the serum was collected and stored at -80° until undergoing analysis.

Detection of cytokines. The Luminex 200 instrument was applied to detect the expression of IFN- γ , IL-10, IL-18 and TNF- α cytokines (eBioscience, EPX340-12167-901). The expression of Human serum sST2 (R&D, CAT# DST200), sCD163 (R&D, CAT# DC163) and Human sCD25/IL-2R (Diacclone, CAT#850.500.096) were measured using ELISA.

Gene sequencing. The exon and related cleavage products of HLH-related genes were obtained by using specific-primer design and PCR on DNA extracted from mononuclear cells. This was followed by bi-directional Sanger sequencing.

Statistical analysis. Statistical analysis was performed using the SPSS19.0 software. All normally distributed data were represented by means \pm standard deviations, and comparison of multiple samples between groups was performed by one-way analysis of variance (ANOVA). All data that were not distributed normally were represented by median and range, and comparison of multiple samples between groups was performed by Wilcoxon rank sum test. *P* values < 0.05 were considered indicative of statistically differences.

Results.

Characteristics of the enrolled participants. Twelve patients diagnosed as pHLH were recruited with an average age of 24 years. The details of the genetic mutations were summarized in **Table 1**.

Table 1. Genetic mutations in the pHLH patients.

| | Gender | Age (years) | Genetic mutation | Mutation loci | Mutation type |
|-----|--------|-------------|------------------|---|---|
| P1 | M | 27 | <i>PRF1</i> | c.G503A:p.S168N c.65delC:p. P22RfsX29 | Heterozygous missense pathogenic variant Heterozygous frameshift pathogenic variant |
| P2 | M | 52 | <i>UNC13D</i> | c.G2588A:p.G863D | Homozygous missense pathogenic variant |
| P3 | F | 31 | <i>ITK</i> | c.A1642C:p.M548L | Heterozygous missense pathogenic variant |
| | | | <i>LYST</i> | c.A1497C:p.K499N | Heterozygous missense pathogenic variant |
| P4 | F | 25 | <i>UNC13D</i> | c.G407A:p.C136Y c.C640T:p.R214X | Heterozygous missense pathogenic variant Heterozygous nonsense pathogenic variant |
| P5 | F | 35 | <i>RAB27A</i> | c. C244T:p.R82C | Homozygous missense pathogenic variant |
| P6 | M | 18 | <i>XIAP</i> | c.894_898del:p.K299LfsX9 | Hemizygous frameshift pathogenic variant |
| P7 | F | 27 | <i>UNC13D</i> | c.G2588A:p.G863D | Homozygous missense pathogenic variant |
| P8 | F | 10 | <i>ITK</i> | c.234+1G>A c. C 85T:p.R29C | Heterozygous missense pathogenic variant Heterozygous missense pathogenic variant |
| P9 | M | 27 | <i>PRF1</i> | c.G503A:p.S168N c.66delG:p.C23AfsX28 | Heterozygous missense pathogenic variant Heterozygous frameshift pathogenic variant |
| P10 | F | 17 | <i>PRF1</i> | c. A 380G:p.N127S c.853-855del:p.K285del | Heterozygous missense pathogenic variant Heterozygous nonframeshift pathogenic variant |
| P11 | F | 18 | <i>PRF1</i> | c. C 46T:p.P16S | Heterozygous |

| | | | | | |
|-----|---|---|-------------|------------------------|---|
| | | | | c. C 1066T:p.R356W | missense pathogenic variant Heterozygous missense pathogenic variant |
| P12 | F | 6 | <i>PRF1</i> | c. C 1349T:p.T450M | Heterozygous missense pathogenic variant |
| | | | | c.853_855del:p.K285del | Heterozygous nonframeshift pathogenic variant |

Seven cases (58.33%) of pHLH have compound heterozygous pathogenic variants, including 5 cases involved *PRF1*, one case involved *UNC13D*, and one case involved *ITK*. Other mutated genes include homozygous pathogenic variants of *UNC13D* in 2 patients (16.67%), homozygous pathogenic variants of *RAB27A* in 1 patient (8.33%), hemizygous pathogenic variants in 1 patient (8.33%), and pathogenic variants in both *ITK* and *LYST* in 1 patient (8.33%).

The clinical symptoms of pHLH patients were characterized by fever, hepatosplenomegaly, hemophagocytosis in bone marrow, and

lymphadenectasis. Fever was observed in all 12 pHLH patients (100%); splenomegaly and hemophagocytosis in bone marrow were found in both 11 patients (91.67%); 9 patients (75%) had lymphadenectasis; Other clinical features included rash (25%), pharyngalgia (8.33%) and arthralgia (16.67%). Besides, five patients (41.67%) had central nervous system involvement. The clinical characteristics of all participants are shown in **Table 2**.

Twenty AOSD associated MAS patients in the active stage were enrolled, including four males and 16 females. The median age of those patients was 26.

Table 2. Characteristics of the enrolled patients (All values are presented as median and range or mean \pm SD).

| Variables | Primary HLH(n=12) | MAS(n=20) | Control group(n=20) |
|------------------------------|------------------------|-------------------------|---------------------|
| Gender (male/female) | 4/8 | 4/16 | 9/11 |
| Age, years (median, range) | 26 (6-52) | 26.5 (18-53) | 37 (22-63) |
| Clinical features (%) | | | |
| Fever | 12/12(100%) | 20/20(100%) | 0 |
| Splenomegaly | 11/12(91.67%) | 14/20(70%) | 0 |
| Lymphadenopathy | 9/12(75%) | 13/20(65%) | 0 |
| Hemophagocytosis | 11/12(91.67%) | 14/20(70%) | ND |
| Rash | 3/12(25%) | 18/20(90%) | 0 |
| Pharyngalgia | 1/12(8.33%) | 8/20(40%) | 0 |
| Arthritis & Arthralgia | 2/12(16.67%) | 14/20(70%) | 0 |
| CNS Syndrome | 5/12(41.67%) | 0/20(0%) | 0 |
| Laboratory variables | | | |
| WBC ($\times 10^9/L$) | 3.30 \pm 2.15 | 9.19 \pm 11.83 | 5.72 \pm 0.85 |
| HGB(g/L) | 99.67 \pm 24.74 | 93.00 \pm 17.01 | 142.10 \pm 11.27 |
| PLT ($\times 10^9/L$) | 107.08 \pm 72.95 | 171.55 \pm 118.64 | 250.8 \pm 56.63 |
| Ferritin (ng/ml) | 1314.25 \pm 932.17 | 3746.6(500.1-55760)★ | ND |
| ALT(U/L) | 54.25 \pm 43.66 | 113.31 \pm 86.85 | 15.95 \pm 7.36 |
| AST(U/L) | 62.33 \pm 77.48 | 109.41 \pm 149.45 | 18.93 \pm 5.83 |
| TBIL (μ mol/L) | 16.71 \pm 11.57 | 35.88 \pm 64.41 | 11.64 \pm 3.77 |
| LDH(U/L) | 596.67 \pm 330.60 | 515.86 \pm 373.00 | 158.45 \pm 22.96 |
| Triglyceride (mmol/L) | 1.95 \pm 1.15 | 2.69 \pm 1.54 | 1.28 \pm 0.71 |
| Fibrinogen (g/L) | 2.16 \pm 1.14 | 2.85 \pm 1.83 | 2.99 \pm 0.73 |
| NK cell activity (%) | 13.10 \pm 3.73 | 14.61 \pm 1.69 | 16.37 \pm 3.38 |
| Soluble CD25 (pg/ml) | 21062.1 \pm 18515.26 | 11074.78 \pm 11149.96 | 795.65 \pm 558.44 |

ND =not done, ★ median and range, n= number.

years. In MAS patients, fever was observed in all 20 patients (100%); splenomegaly and hemophagocytosis in bone marrow were found in both 14 patients (70%), 13 patients (65%) had lymphadenectasis; up to 18 (90%) patients had rash, and the incidence of pharyngalgia and arthralgia was 40% and 70%. No patients suffered central nervous system involvement.

Treatment and outcomes. 8 cases (66.67%) of pHLH received HLH-94/2004 regimen as initial treatment; 4 cases (33.33%) of pHLH received dexamethasone as initial treatment. Three cases (25%) have no response to the initial treatment after two weeks. The DEP regimen²⁵ was used as salvage therapy for those three patients, and all of them (25%) achieved partial response (PR) or complete response (CR). Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) was performed in six patients (50%) within the remission stage. Among the six patients who received Allo-HSCT, two died, and the remaining 4 HLH patients survived. The death of the two patients was attributed to graft-versus-host disease (GVHD) and severe pneumonia, respectively. All of the six patients who did not receive Allo-HSCT relapsed, and four patients died (**Table 3**).

Table 3. Treatment and outcomes of 12 pHLH.

| Initial Treatment | n/12(%) | Outcomes (PR+CR) |
|-------------------|--------------|------------------|
| HLH-94 regimen | 6/12(50%) | 5/6(83.33%) |
| HLH-2004 regimen | 2/12(16.67%) | 2/2(100%) |
| Steroid | 4/12(33.33%) | 2/4(50%) |

PR partial response, CR complete response.

14 cases (70%) of MAS received HLH-94/2004 regimen as initial treatment; 2 cases have no response to this treatment, and one patient died. Three patients (15%) underwent the regime of steroid combined CsA. DEP regimen was carried out in 2 patients (10%). All the five patients achieved PR after two weeks. One patient (5%) died before the initial treatment was performed (**Table 4**).

Different levels of sST2 and sCD163 in pHLH and MAS. In order to identify the potential markers of cytokines in HLH, we analyzed the levels of sST2 in the pHLH, MAS and control groups (80.75 ± 87.04 ng/ml, $363.13 \pm$

Table 4. Treatment and outcomes of 20 MAS.

| Treatment | n/20(%) | Outcomes (PR+CR) |
|----------------------|-----------|------------------|
| HLH-94 regimen | 9/20(45%) | 7/9(77.78%) |
| HLH-2004 regimen | 5/20(25%) | 4/5(80%) |
| DEP regimen | 2/20(10%) | 2/2(100%) |
| Steroid combined CSA | 3/20(15%) | 3/3(100%) |
| Other treatment | 1/20(5%) | 0/1(0) |

PR partial response, CR complete response.

307.24 ng/ml and 19.05 ± 8.31 ng/ml, respectively), as shown in **Figure 1A**, it appears that levels of sST2 were significantly increased in MAS patients in comparison with that in pHLH ($P = 0.004$) and healthy controls ($P = 0.001$). Next, sCD163 levels in pHLH, MAS and healthy controls were detected and analyzed. Median sCD163 levels in patients with MAS were higher compared with patients with pHLH and were elevated compared with healthy controls (3532.72 ± 2479.68 ng/ml, 1731.96 ± 1262.07 ng/ml and 393.94 ± 148.72 ng/ml, respectively). Strikingly, statistically significant differences of sCD163 levels were also observed between the pHLH and MAS groups ($P = 0.046$) (**Figure 1B**).

Comparison of serum levels of IFN- γ , IL-10, IL-18 and TNF- α among groups of patients pHLH, MAS and healthy control. To explore the role of IFN- γ , IL-10, IL-18, and TNF- α in the development of HLH, we measured and compared the expressions of those cytokines in the groups. In patients with pHLH and MAS, there was a trend toward higher levels of IFN- γ , IL-10, IL-18 and TNF- α than that in healthy controls (306.89 ± 281.60 pg/ml, 20.40 ± 30.49 pg/ml, 463.33 ± 597.04 pg/ml, 61.48 ± 84.69 pg/ml in pHLH; 562.43 ± 399.86 pg/ml, 8.3 ± 13.14 pg/ml, 1247.82 ± 1318.58

pg/ml, 106.10 ± 77.21 pg/ml in MAS, respectively). IL-10 could not be detected in the majority of healthy subjects, and a barely detectable concentration of IL-10 was observed in only three healthy participants (0.11 pg/ml, 0.11 pg/ml and 6.19 pg/ml). TNF- α could be detected in 4 healthy persons (22.94 pg/ml, 40.37 pg/ml, 9.06 pg/ml and 5.22 pg/ml) and IFN- γ could be detected in only one healthy serum sample (2.14 pg/ml). Similarly, IL-18 levels are also difficult to detect in the serum of healthy group, and we only detected the expression of IL-18 in 7 persons (16.79 pg/ml, 70.71 pg/ml, 69.26 pg/ml, 43.38 pg/ml, 2.71 pg/ml, 4.82 pg/ml and 6.72 pg/ml, respectively). These data clearly show a cytokine storm in pHLH, MAS with raised levels of IFN- γ , IL-10, IL-18, and TNF- α but no significant differences were found between pHLH and MAS (**Figure 2**).

sCD25. In patients with pHLH and MAS, the level of sCD25 before initial treatment was significantly increased compared to healthy controls (21062.1 ± 18515.26 pg/ml in pHLH, 1074.78 ± 11149.96 pg/ml in MAS, and 795.65 ± 558.44 pg/ml in control), but there was no a significant difference between pHLH and MAS groups ($P = 0.07$). Two weeks after treatment, we found an obvious decline in the expression of sCD25 in both pHLH and MAS groups (21062.1 ± 18515.26 pg/ml vs 9835.98 ± 4015.52 pg/ml, 11074.78 ± 11149.96 pg/ml vs 1766.88 ± 1358.93 pg/ml, respectively). This result may indicate that sCD25 is

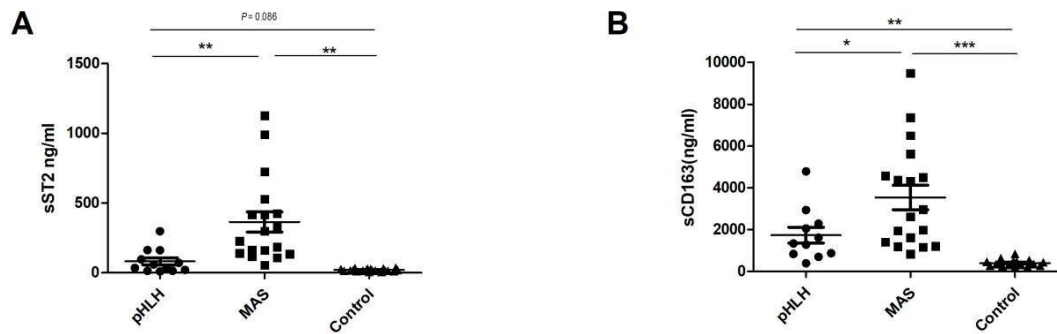


Figure 1. Expression levels of sST2 and sCD163 among the groups of pHLH, MAS and controls. Serum level (ng/ml) of sST2; (B) Serum level (ng/ml) of sCD163. The expression of sCD163 in one pHLH patient and the expression of sCD163 and sST2 in two MAS patients were not available. The horizontal line represents the quartile and the median. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

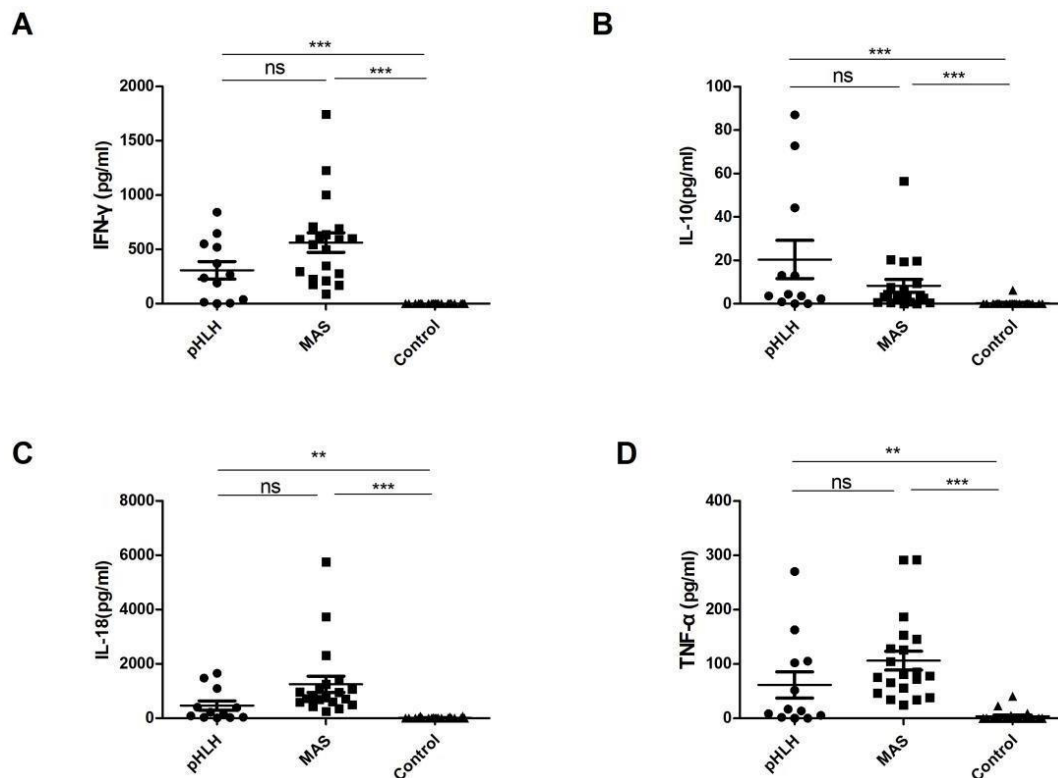


Figure 2. Serum levels of IFN- γ , IL-10, IL-18 and TNF- α in pHLH, MAS patients and control group. (A) Serum levels (pg/ml) of IFN- γ ; (B) Serum levels (pg/ml) of IL-10; (C) Serum levels (pg/ml) of IL-18; (D) Serum levels (pg/ml) of TNF- α . Expression levels of cytokines undetectable were represented by 0. The horizontal line represents the quartile and the median. ns not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

closely associated with the activity of the disease (Figure 3).

Discussion. pHLH, a severe subtype of HLH, is triggered by genetic mutations that induce dysfunctions of NK and cytotoxic T lymphocytes (CTLs). The remaining NK cells and CTLs can't eradicate the antigens effectively. Thus persistent antigen presentation leads to the over-activation of CTLs. The excessive cellular activation and expansion induce macrophages to release large amounts of inflammatory factors, causing a cytokine storm.²⁶ Hitherto, hematopoietic stem cell transplantation (HSCT) is the

optimal treatment for pHLH.^{15,27-29} MAS, as previously discussed, is categorized as a form of secondary HLH.⁵ The development of MAS is also featured by a cytokine storm, with the presentation of numerous proinflammatory cytokines. Hence, pHLH and MAS bears great similarity in the cytokine profiles.

Accumulated evidence suggests that many cytokines play a pivotal role in the pathogenesis of HLH. In our study, we initially investigated the expression of sST2, sCD163, sCD25, MIP-1 α , SDF-1 α , IL-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, IL-1 RA, RANTES, IFN- γ ,

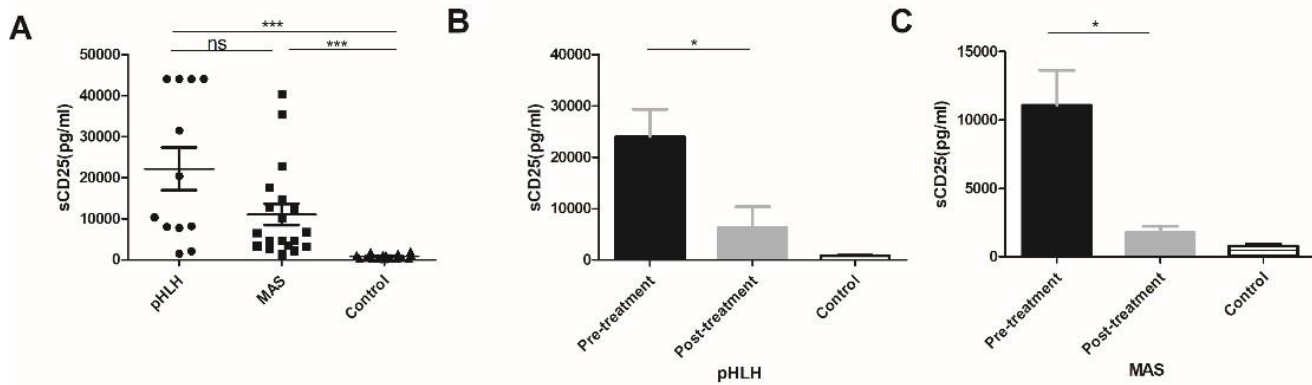


Figure 3. Serum levels of sCD25 in pHLH, MAS patients and control group. Serum levels of sCD25 in pHLH, MAS patients before treatment and control group. Serum levels of sCD25 in pHLH before and after treatment. Serum levels of sCD25 in MAS before and after treatment. The horizontal line represents the quartile and the median. ns: not significant; * $P < 0.05$; *** $P < 0.001$.

GM-CSF, TNF- α , MIP-1 β , IFN- α , MCP-1, TNF- β , GRO- α and Eotaxin in pHLH, MAS and healthy group. The majority of those cytokines, however, did not affect the diagnosis and differential diagnosis of HLH.

IFN- γ is considered to be uniquely essential for the development of HLH and evidence suggests that neutralization of IFN- γ has a dramatic effect on the survival of mice with HLH.¹⁷ IL-10 is generally thought to be an immunosuppressive molecule and has a protective effect on inflammatory responses. However, excessive IL-10 may accelerate the progression of HLH via inhibiting activations of lymphocytes which are crucial for immune homeostasis.¹⁸ In line with this, the study of Xiao-Jun²¹ implies that the elevated levels of IFN- γ and IL-10 with only modestly elevated IL-6 has high diagnostic accuracy for HLH. IL-18 is also involved in the pathophysiology of HLH and Takada H¹⁹ reveals that IL-18 levels significantly correlate with the activity of HLH. TNF- α has long been illustrated to be associated with the occurrence and development of many diseases. Henter²⁰ demonstrates that TNF levels are augmented in active FHL and may contribute to the pathogenesis of the disease. So, accumulated evidence indicates that those cytokines are of paramount importance in HLH. In our study, the expressions of all those cytokines increase in both pHLH and MAS groups compared to the healthy group, which is consistent with the above-published studies. Interestingly, there is no significant difference in the expressions of those cytokines between pHLH and MAS, indicating that the levels of IFN- γ , IL-10, IL-18 and TNF- α cannot contribute to the differentiation of pHLH and MAS.

We further analyzed the cytokines with different expression levels in pHLH and MAS. sCD163 is the soluble form of CD163, which acts as the hemoglobin-haptoglobin scavenger receptor. CD163 expresses mainly on the membranes of activated monocytes/macrophages and is regarded as a macrophage-specific marker for inappropriate macrophage activation in inflammatory diseases.^{23,30} Upregulation of CD163 on the surface of activated

monocytes or macrophages can facilitate the process of phagocytosis; therefore some scholars consider it as a potential biomarker for HLH and relevant diseases.²³ ST2, expressed in Th2 cells, belongs to the IL-1 receptor family and is a receptor of IL-33. ST2 can express as a membrane-bound form (ST2L) or a secreted form (sST2) and has been clearly implicated as a regulator of both the development and effect phases of Th2-type responses.³¹ Th2 cells mainly secrete IL-4, IL-5 and IL-13, and those cytokines regulate immune response by activating B cells to produce antibodies or by deactivating and reprogramming macrophages.³¹⁻³³ sST2 can mediate this response through downregulating the pro-inflammatory effect of macrophages.^{31,34} Previous studies have shown that the expression of sST2 elevated in systemic juvenile idiopathic arthritis and MAS.³⁵ And the blockage of the sST2 pathway can facilitate the treatment of HLH in Perforin deficient mice.

Serum soluble interleukin-2 receptor (sCD25) is the most studied cytokine/cytokine receptor to date in HLH and is one of the diagnostic criteria set in the HLH-2004 criterion. In our study, we found that the level of sCD25 is higher in both pHLH and MAS group compared to the control group, but no significant differences were found between pHLH and MAS groups. Moreover, the level of sCD25 decreased when the patients received treatment. This data indicates the importance of sCD25 as a diagnostic and disease marker in HLH (including MAS).^{36,37}

Conclusions. In our study, sCD163 and sST2 levels in pHLH were significantly lower than that in MAS. We suggest that sST2 and sCD163 may serve as markers to distinguish pHLH from MAS. The elevated sCD163 levels in MAS patient may indicate that the macrophage activation in MAS is higher than that in pHLH. Furthermore, we speculate that sST2 may act as a counterbalance to the over-activated macrophages and, consequently, the over-activation of macrophage in MAS results in the higher levels of sST2. Nevertheless,

the specific molecular mechanism for the elevated sST2 levels in MAS needs to be further investigated. The elevated levels of IFN- γ , IL-10, IL-18, TNF- α , and sCD25 can contribute to the diagnosis of HLH, but may not discriminate pHLH from MAS. Levels of sST2 and sCD163 in MAS were significantly higher than that in pHLH, sST2 and sCD163 may serve as markers to distinguish pHLH from MAS.

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