Human Immunology 80 (2019) 1006–1011

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

Evaluation of lymphocyte function by IFN-γ secretion capability assay in the diagnosis of lymphoma-associated hemophagocytic syndrome

Hongyan Hou[a], Ying Luo[a], Feng Wang[a], Jing Yu[a], Dengju Li[b,*], Ziyong Sun[a,*]

[a] Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Jiefang Road 1095#, Wuhan, China
[b] Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Jiefang Road 1095#, Wuhan, China

ARTICLE INFO

Keywords:
Lymphoma
Lymphoma-associated hemophagocytic syndrome (LAHS)
Lymphocyte function
Diagnosis

ABSTRACT

Lymphoma-associated hemophagocytic syndrome (LAHS) is a highly life-threatening disease characterized by an uncontrolled immune disorder. Both under-recognition and delayed diagnosis may contribute to aggressive diseases, and a poorer prognosis. Identification of laboratory features specific for LAHS patients may allow for early detection and intervention with improved outcomes. In the present study, 120 lymphoma patients at first diagnosis were recruited and the function of lymphocytes was evaluated by IFN-γ secretion assay at first diagnosis and follow up. During the surveillance period, 20 patients who complicated with hemophagocytic lymphohistiocytosis (HLH) were classified as LAHS group, and 30 patients without infectious diseases during the course of treatment were classified as lymphoma control group. In addition, 20 non-malignant associated HLH patients recruited as HLH control group and 50 healthy control (HC) subjects were also included. The IFN-γ secretion capability of lymphocytes was compared between first diagnosis of lymphoma patients who was complicate with HLH or not in the disease progression. Our results showed that only NK cell activity was decreased in lymphoma control group, but the activities of NK, CD4+ and CD8+ T cells were all significantly decreased at the time of lymphoma diagnosis in those who would progress with HLH. During the course of treatment, lymphocyte function was relatively stable in lymphoma patients but became further decreased when suffering from complication of LAHS. The IFN-γ secretion capability of lymphocytes in LAHS and non-malignant associated HLH patients were all significantly decreased compared with HCs. So the occurrence of HLH was the key factor leading to the impaired activity of lymphocytes. These data suggest that decreased lymphocyte function might be used as a predictor of LAHS, which has critical clinical significance in diagnosis and further understanding the pathogenesis of the disease.

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH), also called hemophagocytic syndrome is a potentially life-threatening disease characterized by impaired natural killer (NK) and cytotoxic T cell function, cytokine storm and overwhelming inflammation [1]. The clinical syndrome of HLH is characterized by fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia, hyperferritinemia and hemophagocytosis in haemopoietic organs [2]. Primary HLH patients usually have a family history of this disease or known underlying genetic defects [3]. Secondary or acquired HLH can be triggered by a series of causes, including infection with agents such as Epstein-Barr virus (EBV), malignancies, autoimmune diseases, metabolic diseases and acquired immune deficiencies, such as AIDS and transplantation-associated immunosuppression [4].

Lymphoma is the most common underlying condition in malignancy-associated HLH. The most common types of lymphoma-associated HLH (LAHS) are NK/T cell lymphoma, followed by B cell lymphoma [5,6]. LAHS is considered to be a fatal disease, with the median survival time for patients with LAHS being only 36 days [6]. HLH presents a significant challenge to clinicians due to the lack of identification of underlying causes of the disease and the variable overlaps of symptoms with other diseases.

A diagnosis of HLH is generally made when a patient meets five or

Abbreviations: HLH, Hemophagocytic lymphohistiocytosis; HPS, hemophagocytic syndrome; NK, natural killer; LAHS, lymphoma-associated HLH; EBV, Epstein-Barr virus; LDH, lactate dehydrogenase; HCs, healthy controls

* Corresponding authors at: Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Jiefang Road 1095#, Wuhan 430030, China (D. Li). Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Jiefang Road 1095#, Wuhan 430030, China (Z. Sun).

E-mail addresses: lidengju@163.com (D. Li), zysun@tjh.tjmu.edu.cn (Z. Sun).

https://doi.org/10.1016/j.humimm.2019.09.003
Received 11 May 2019; Received in revised form 30 July 2019; Accepted 3 September 2019
Available online 17 September 2019
0198-8859/

more criteria from a list of eight nonspecific criteria comprising fever, cytopenia, hypofibrinogenemia/hypertriglyceridemia, splenomegaly, hemophagocytosis, hyperferritinemia, elevated soluble interleukin-2 receptor a (sIL-2Ra) and decreased NK cell activity [1]. The HScore which contained 3 clinical, 5 biologic and 1 cytologic variables was used to estimate an individual's risk of having reactive hemophagocytic syndrome [7]. Highly elevated ferritin levels also displayed high sensitivity and specificity for diagnosing HLH [8]. Elevated levels of cytokines (IFN-γ, IL-6, IL-10) and chemokines (MCP-1 and IP-10), which are secreted by Th1 cells, Th2 cells, macrophages, cytotoxic T cells and NK cells, have been reported at the onset of HLH and could be used as a prognostic factor for this disease [9–11]. Although these parameters have been accepted as a highly useful diagnostic tool, several limitations still exist. For example, hemophagocytosis is usually absent at the onset of HLH [12]. In addition, NK cell function data may be unavailable due to the lack of standard detection methods carried out in daily clinical practice or poor peripheral blood cell counts during conditioning chemotherapy. Therefore, to improve early diagnosis and treatment, it is possibly important to identify the clinical and laboratory features specific to HLH.

Previous studies have developed flow cytometry assays by detecting the cytotoxicity and CD107a degranulation of NK cells, which have critical clinical application in the diagnosis of HLH [13,14]. However, no standard methods have been applied in clinical practice. Moreover, other lymphocyte subsets, such as CD4+ T cells and CD8+ T cells are also involved in the pathogenesis of HLH. It has been reported that there was a reduction in CD4+ T cell count and abnormal activation of CD8+ T cells in EBV-HLH patients [15], so it is also critical to explore the function of T cells in the development of HLH. In our previous studies, we have successfully developed a simple and rapid method to evaluate the function of NK, CD4+ and CD8+ T cells simultaneously by IFN-γ secretion assay in whole blood after stimulation with PMA/ionomycin. Accordingly, we have established reference intervals of lymphocyte function in healthy adults, which has important clinical value in the diagnosis, monitoring and prognosis of immune-related diseases [16]. Therefore, we aimed to detect lymphocyte activity by our previously established method which could be applied easily in clinical practice and might have potential clinical significance in the early diagnosis of HLH.

In this study, we detected the IFN-γ secretion capability of lymphocytes in lymphoma patients at first diagnosis and follow up. During surveillance, the IFN-γ secretion capability of lymphocytes at first diagnosis was compared with that at onset of LAHS or with that of infection-free lymphoma patients. The diagnostic value of lymphocyte function was investigated in LAHS.

2. Materials and methods

2.1. Study subjects

This was a prospective study, approved by the ethical committee of Tongji hospital, Tongji Medical College, Huazhong University of Science and Technology. From January 2017 to October 2018, 120 consecutive lymphoma patients were recruited according to the World Health Organization 2016 classification of hematopoietic and lymphoid tumors [17]. Each patient was monitored in the next one year. In the follow up, the patients who complicated with HLH and without infectious diseases were diagnosed as LAHS. The diagnosis of HLH was referred to Histiocytosis Society- 2004 [2]. To strengthen the diagnosis of HLH, HScore were calculated using the Fardet algorithm at the time of HLH onset [7]. The median HScore was 218 (range, 192–243) and the probability of having HLH was 97%. The patients who present up front with HLH and family HLH with gene mutation were excluded. Patients who didn't complicate with any infection during the course of treatment were classified as the lymphoma control group. At last, 20 LAHS patients and 30 lymphoma control patients were included. In addition, 20 non-malignant associated HLH patients were recruited as HLH control group (including 15 EBV infection and 5 autoimmune diseases). Fifty healthy controls were also recruited and determined by interview and physical examination. All subjects gave written informed consent.

2.2. Measurements of lymphocyte function

The lymphocyte function of the patients was evaluated at first diagnosis of lymphoma. During the course of treatment, the lymphocyte function was re-evaluated every three months in the next one year (0, 3, 6, 9 and 12 month). The lymphocyte function was also detected at time point that the patients were suspected the onset of HLH. The evaluation of lymphocyte function should be completed before chemotherapy at each time point.

Lymphocyte function was detected based on IFN-γ secretion assay upon PMA/ionomycin stimulation according to our previous studies [16] and the panel of fluorescent antibodies (listed in the following text) was further modified in the flow cytometry assay. Heparinized venous blood was collected before using chemotherapy drugs or glucocorticoid and was assessed within 6 h. Whole blood was diluted with IMDM medium (Gibco-BRL, Grand Island, NY, USA) and stimulated by Leukocyte Activation Cocktail with BD GolgiPlug™ (including 50 ng/ml PMA, 1 μM ionomycin and 1 μg/ml brefeldin A, BD Biosciences, San Jose, CA, USA) for 4 h at 37 °C with 5% CO_2. Samples were stained with anti-CD4-APC/H7, anti-CD3-FITC, anti-CD8-PE, anti-CD45-PerCP/Cy5.5 and anti-CD56-PE/Cy7 (BD Biosciences, San Jose, CA, USA), followed by fixation/permeabilization and staining with anti-IFN-γ-APC (BD Biosciences San Jose, CA, USA). Flow cytometric analysis was performed with a BD Biosciences FACSCanto II flow cytometer.

2.3. Statistical analysis

Differences between groups of participants were analyzed using the Mann-Whitney *U* test. Differences of lymphocyte activity of one patient between different time points in LAHS and lymphoma control groups were analyzed using paired t-tests. Statistical analyses were performed using GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA). Statistical significance was determined as $p < 0.05$. ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

3. Results

3.1. Patient characteristics

In this study, 20 LAHS patients, 30 lymphoma control patients, 20 non-malignant HLH patients and 50 healthy controls were recruited. Demographic data, routine blood cell counts and the level of lactate dehydrogenase (LDH) are shown in Table 1. White blood cell, neutrophil, lymphocyte and platelet counts were significantly decreased but LDH levels were significantly elevated in LAHS and non-malignant HLH groups. Table 2 presents the subtypes and stages of lymphoma. In the lymphoma control group, the main lymphoma types were diffuse large B cell (n = 13, 43.3%), follicular (n = 6, 20%), NK/T cell (n = 9, 30%) and anaplastic large cell lymphoma (n = 2, 6.7%). In the LAHS group, the most common types of lymphoma were NK/T cell lymphoma (n = 8, 40%), followed by diffuse large B cell (n = 7, 35%), follicular (n = 3, 15%) and anaplastic large cell lymphoma (n = 2, 10%). (See Table 2).

3.2. Comparison of lymphocyte function in lymphoma patients who would progress with HLH or not

To compare the difference of lymphocyte function between first diagnosis of lymphoma patients who would complicate with HLH or not in the disease progression, IFN-γ secretion capability of lymphocytes

Table 1
Basic characteristic of the lymphoma and HLH patients.

Parameters	HCs	Lymphoma control group	LAHS	non-malignant HLH
Total (Male)	50(25)	30(15)	20(10)	20(10)
Age	49.32 ± 2.332	50.36 ± 3.018	52.27 ± 3.110	38.23 ± 2.543
WBC (10^9/L)	6.221 ± 0.983	6.555 ± 0.894	2.032 ± 1.213	3.245 ± 1.032
Neut (10^9/L)	3.533 ± 0.383	3.861 ± 0.699	1.232 ± 0.564	1.834 ± 0.742
Lym (10^9/L)	1.732 ± 0.276	1.631 ± 0.367	0.353 ± 0.232	0.753 ± 0.352
Plt (10^9/L)	168.2 ± 12.12	161.5 ± 18.19	42.22 ± 10.21	31.92 ± 9.23
LDH (U/L)	230.8 ± 35.22	351.5 ± 39.56	1655 ± 130.2	883.2 ± 86.39

HCs: healthy controls; LAHS: Lymphoma-associated hemophagocytic syndrome; HLH: hemophagocytic lymphohistiocytosis; Neut: neutrophil; Lym: lymphocyte; Plt: platelet; LDH: Lactate dehydrogenase; Data were shown as mean ± SEM.

Table 2
The distribution of subtypes in lymphoma control and LAHS groups.

Underlying diseases	No. of patients %	Stages
Lymphoma	30	
Diffuse large B-cell lymphoma	13 (43.3%)	IVB (10), IIIB (3)
Follicular lymphoma	6 (20%)	IVB (5), IIIB (1)
NK/T cell lymphoma	9 (30%)	IVB (9)
Anaplastic large cell lymphoma	2 (6.7%)	IVB (2)
LAHS	20	
NK/T cell lymphoma	8 (40%)	IVB (8)
Diffuse large B-cell lymphoma	7(35%)	IVB (7)
Follicular lymphoma	3 (15%)	IVB (3)
Anaplastic large cell lymphoma	2 (10%)	IVB (2)

LAHS: Lymphoma-associated hemophagocytic syndrome.

was evaluated (Fig. 1A, B). The NK cell activity was lower in lymphoma control group than HCs, but the activities of NK, CD4+ and CD8+ T cells were all significantly decreased at the time point of lymphoma diagnosis in lymphoma patients who would progress with HLH (Fig. 2A–C). These data suggested that the decreased IFN-γ secretion capability of NK and T cells might be used as risk indicators of HLH in lymphoma patients.

3.3. Monitoring the lymphocyte function of lymphoma patients

The IFN-γ secretion capability of lymphocytes in lymphoma patients was analyzed during the surveillance period. The results show that the activity of NK, CD4+ and CD8+ T cells was relatively stable in lymphoma control group within one year but significantly decreased in LAHS patients from diagnosis of lymphoma to LAHS occurrence (Fig. 3A–C). The expression of HLA-DR on CD4+ and CD8+ T cells and the expression of CD56 on NK cells were also analyzed. T cells displayed an activated phenotype but the percentages of $CD56^{high}$ and $CD56^{dim}$ NK cells were decreased in LAHS group (Fig. 3D–F). Furthermore, the IFN-γ secretion capability of lymphocytes was significantly decreased in both LAHS and non-malignant HLH groups when compared with that of healthy controls, but no significant differences were observed between the two groups (Fig. 4A–C). The lymphocytes displayed an active phenotype due to the over-activation in vivo, but the IFN-γ secretion capability was reduced followed by PMA/ionomycin stimulation. So impaired lymphocyte function was one major clinical characteristic of HLH. Table 3 shows the lymphocyte function of 4 representative lymphoma patients from first diagnosis to the onset of HLH. The NK cell activity of these four patients was decreased in all cases. In patients 2 and 3, CD4+ T cells exhibited decreased activity. In patients 2 and 4, decreased CD8+ T cell activity was observed (Table 3).

4. Discussion

HLH is one of the most critical clinical conditions with a mortality rate reported in large series ranging between 42% [18]and 75% [4]. The survival data from several studies were approximately 56%–70% and the median overall survival time was 36–230 days [1]. Lymphoma is the most common underlying condition of malignancy-associated HLH. The median survival time for patients with NK/T cell lymphoma and other types of T cell lymphoma is only 28 and 33 days, respectively [19]. In general, in the absence of timely and effective treatment, patients experience a rapid and fatal course of disease. Early diagnosis of underlying condition, especially of lymphoma, contributes to better outcomes. However, misdiagnosis of LAHS often occurs because of the lack of lymphadenopathy or mass formation amenable to biopsy, which further contributes to the development of progressive disease with an unfavorable prognosis. Therefore, the identification of clinical and laboratory characteristics of LAHS in adults may allow for early detection and intervention with improved outcomes.

Although several clinical features of LAHS have been reported, there is no consensus for LAHS diagnosis. Multivariate logistic regression analysis shows that younger age, bone marrow involvement and reduced serum albumin were independent risk factors for developing HLH in extranodal NK/T-cell lymphoma patients [20]. Hepatosplenomegaly, increased LDH and ferritin, fibrinogen and platelet count have also been reported to be risk factors for LAHS [21–23]. Moreover, hypercytokinemia is a major pathologic feature of HLH, caused by hyperactivation of cytotoxic T cells and macrophages. Previous studies have shown that active HLH had elevated IL-10, IFN-γ and IL-6 [9,24]. Increased IL-6 and IL-10 levels could be used as independent factors for predicting LAHS [21]. High serum levels of IL-10 and IFN-γ were also reported to be associated with early death of HLH [25], and IFN-γ and IL-18 were thought to be sensitive indicators of disease activity [26–28]. Currently, lymphoma is one of the most prevalent hematological diseases and the incidence of LAHS is also increasing. However, there are few studies to compare the clinical characteristics and host immune status of lymphoma and LAHS, which could further explore predictors for development of LAHS and improve understanding of the pathogenesis of this disease.

To accurately measure NK cell activity has become increasingly important as the updated diagnostic guidelines for HLH include low or absent NK cell activity. Previous studies have exported a series of methods to evaluate NK cell activity, such as the CD107a degranulation and cytotoxicity assay, which could be used for evaluation of immune parameters in patients with HLH [13,29]. However, T cells are also involved in the development of HLH; abnormal activity of CD4+ and CD8+ T cells also contribute to the excessive inflammation observed [15]. In our previous studies, we have established a simple method to evaluate NK, CD4+ and CD8+ T cells simultaneously by IFN-γ secretion assay using flow cytometry. Our results show that only the NK cell activity was decreased in lymphoma patients compared with healthy controls, but the activities of the three populations were all decreased at the time of lymphoma diagnosis in patients who would suffer from complication of HLH. So the decreased activity of lymphocytes might be one risk marker for HLH occurrence in lymphoma patients. Furthermore, we found that the IFN-γ secretion capability of lymphocytes was relatively stable in lymphoma patients who did not suffer any infection during follow up. Although T cells displayed an activated phenotype with high HLA-DR expression, the IFN-γ secretion capability of both T

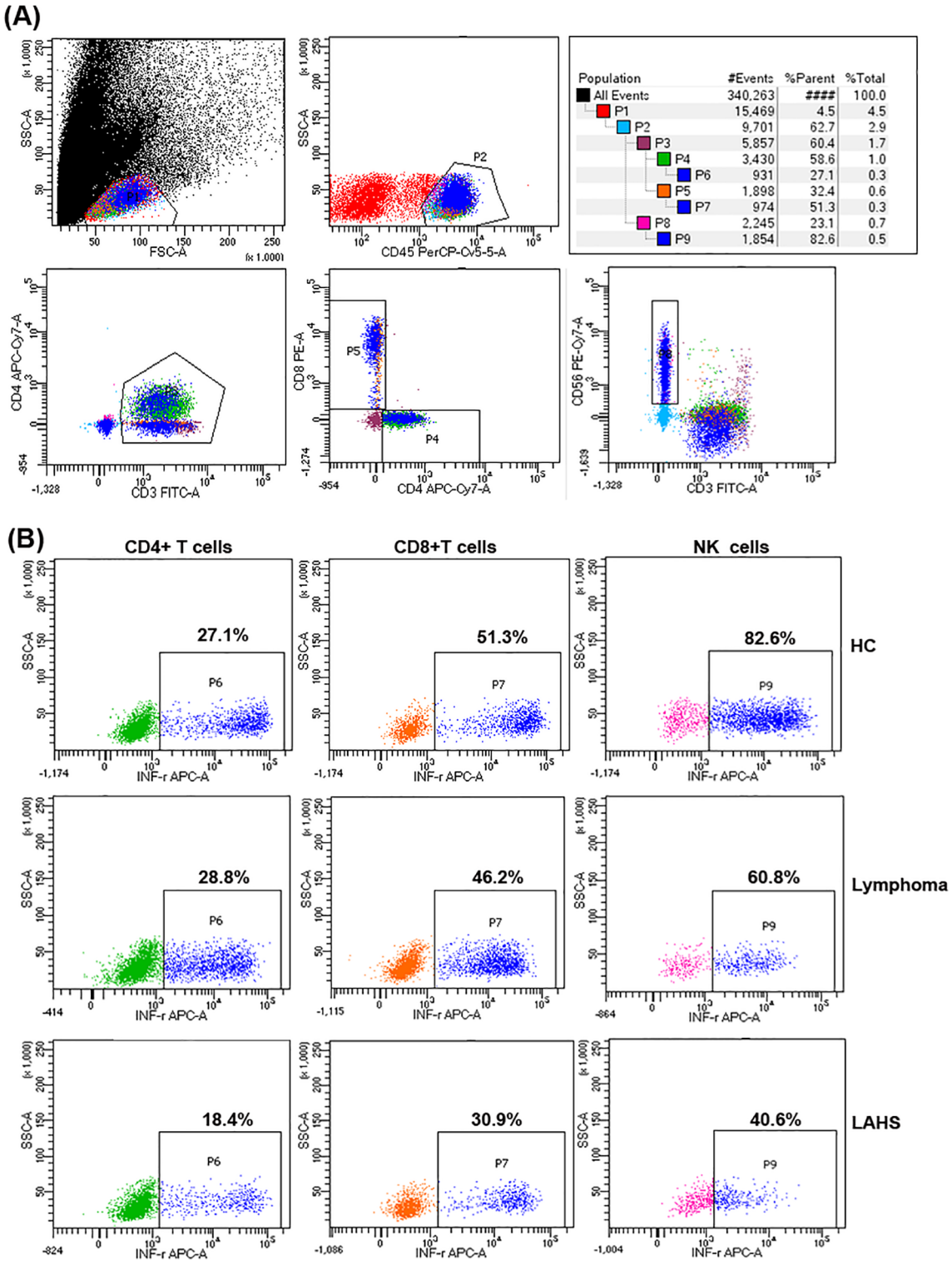

Fig. 1. Determination of lymphocyte function by IFN-γ secretion assay. Diluted whole blood (1:5) was stimulated by Leukocyte Activation Cocktail with BD GolgiPlug™ for 4 h. Intracellular staining of IFN-γ was performed by flow cytometry. (A) Representative flow cytometry gating strategy for identification of NK, CD4 + and CD8 + T cells. (B) Representative dot plots show the expression of IFN-γ by CD4 + and CD8 + T cells and NK cells in healthy adults (HC), lymphoma and LAHS patients.

cells and NK cells was significantly decreased when LAHS occurred. The impaired lymphocyte function was also observed in non-malignant HLH group. These data suggest that decreased lymphocyte function might be used as a predictor of HLH and have potential value in the clinical diagnosis of LAHS.

This study had several limitations. Firstly, the composition of lymphoma subtypes between the LAHS and lymphoma control groups were a little different. Secondly, we did not systematically analyze the relationship between pathologic characteristics of lymphoma and lymphocyte function. Therefore, large-scale and well-designed studies with both clinical and molecular biomarkers are warranted to better clarify the prognostic value of lymphocyte function in LAHS.

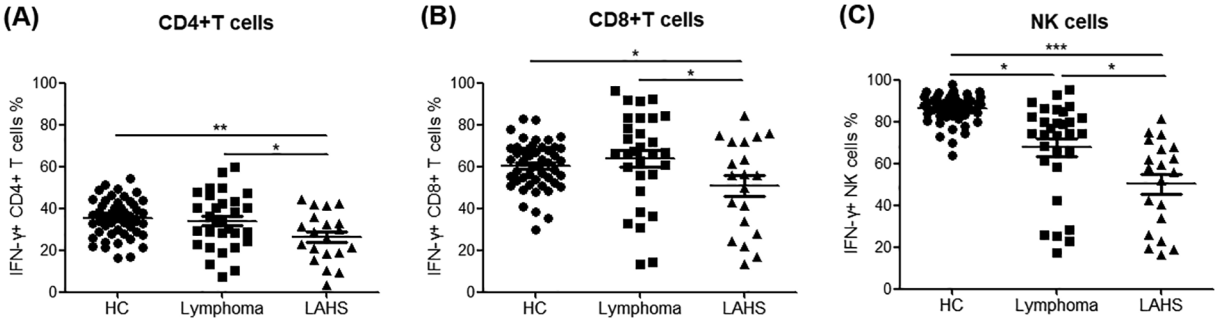

Fig. 2. Lymphocyte function in lymphoma patients who would complicate with HLH or not in the disease progression. The lymphocyte function of 30 lymphoma control patients and 20 lymphoma patients who would complicate with HLH in the progression (LAHS group) at the time of first diagnosis of lymphoma, and 50 healthy adults were detected. Diluted whole blood was stimulated with Leukocyte Activation Cocktail BD GolgiPlug™ for 4 h. The production of IFN-γ in NK, CD4+ and CD8+ T cells were analyzed by flow cytometry. Scatter plots showing the percentages of IFN-γ+ cells in (A) CD4+ T cells, (B) CD8+ T cells and (C) NK cells. Data are shown as mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

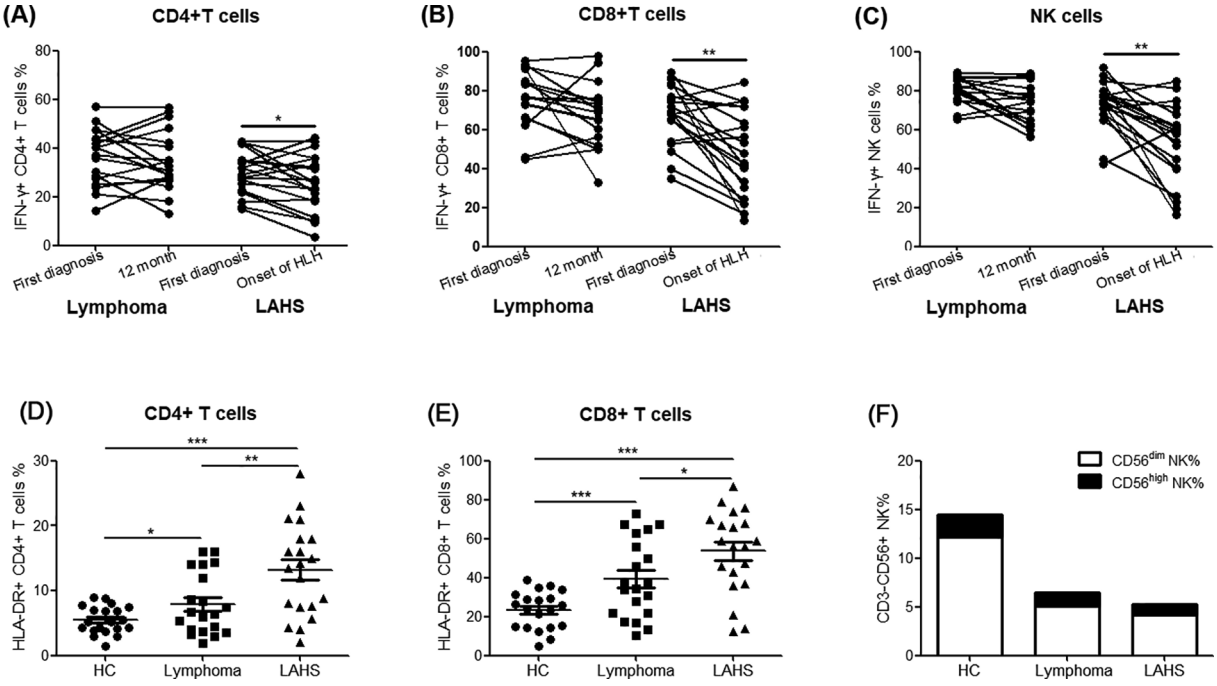

Fig. 3. Lymphocyte function in lymphoma patients suffering complication of LAHS. The IFN-γ secretion capability of lymphocytes was detected in lymphoma patients at first diagnosis and during follow up. The IFN-γ secretion capability of lymphocytes in lymphoma control group at first diagnosis was compared with that at 12 months after treatment. In the LAHS group, the IFN-γ secretion capability of lymphocyte was compared between the first diagnosis of lymphoma and the onset of LAHS. Scatter plots showing the percentages of IFN-γ+ cells in (A) CD4+ T cells, (B) CD8+ T cells and (C) NK cells. The expression of HLA-DR on (D) CD4+ T cells, (E) CD8+ T cells and (F) NK cells in HCs, lymphoma and LAHS groups was shown. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

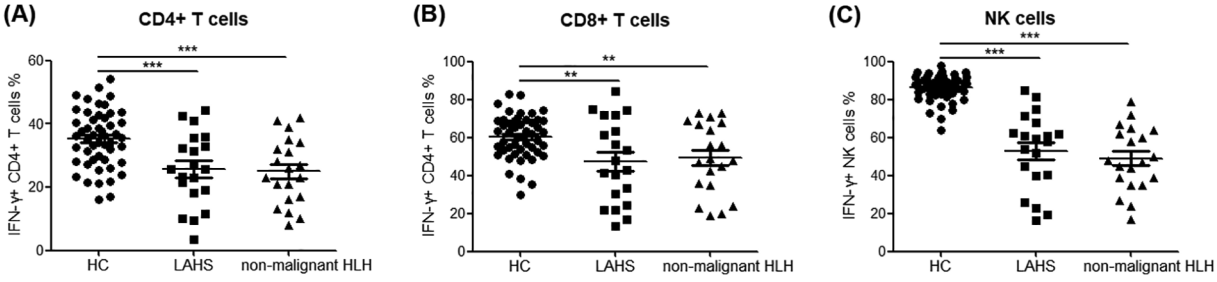

Fig. 4. Lymphocyte function in HLH patients. The IFN-γ secretion capability of lymphocytes in non-malignant HLH patients was detected. The percentages of IFN-γ+ cells in (A) CD4+ T cells, (B) CD8+ T cells and (C) NK cells from HCs, LAHS and non-malignant HLH patients were shown. $**p < 0.01$, $***p < 0.001$.

In conclusion, this is the first study to analyze lymphocyte function in lymphoma and LAHS patients, and monitor the trend of lymphocyte activity change from first diagnosis of lymphoma to the development of LAHS. The results suggest that lymphocyte function, including that of NK, CD4+ and CD8+ T cells, was significantly decreased in LAHS patients. This study has critical clinical significance in further understanding the pathogenesis of LAHS and could be used as a predictive factor in the diagnosis of this disease.

Table 3
Lymphocyte function changes in lymphoma patients complicated with LAHS.

	Lymphoma	IFN^+CD4^+ T cells%	IFN^+CD8^+ T cells%	IFN^+NK cells%
Patient1	NK/T cell lymphoma			
Stage	IVB			
IPI	3			
1 month		42.5	71.8	62.6
3 month		40.5	67.1	60.0
5 month ((HLH onset*)		55.2	73.3	25.8
Patient2	Diffuse large B cell lymphoma			
Stage	IVB			
IPI	4			
1 month		25.4	55.6	84.8
3 month		22.8	65.1	78.8
6 month (HLH onset*)		11.5	33.4	54.4
Patient3	T-lymphoblastic lymphoma			
Stage	IVB			
IPI	2			
1 month		19.5	63.4	79.3
3 month		22.2	83.8	84.7
6 month		35.4	78.4	86.6
7 month* (HLH onset*)		21.8	81.3	47.9
Patient4	Diffuse large B cell lymphoma			
Stage	IVB			
IPI	4			
1 month		41.8	89.2	73.5
3 month		39.9	92.3	92.6
6 month		34.1	84.8	87.2
9 month		38.1	78.5	74.5
11 month (HLH onset*)		31.4	41.5	16.4

LAHS: Lymphoma-associated hemophagocytic syndrome. *: the time point when the physicians suspected the onset of HLH and the lymphocyte IFN-γ secretion capability was detected.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Infectious Diseases Control Project from Ministry of Health of China (2016ZX10004207-004) and National Natural Science Foundation of China (81770168).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.09.003.

References

[1] M. Ramos-Casals, P. Brito-Zeron, A. Lopez-Guillermo, M.A. Khamashta, X. Bosch, Adult haemophagocytic syndrome, Lancet 383 (2014) 1503.
[2] J.I. Henter, A. Horne, M. Arico, R.M. Egeler, A.H. Filipovich, S. Imashuku, et al., HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis, Pediatr. Blood Cancer 48 (2007) 124.
[3] S.W. Canna, E.M. Behrens, Not all hemophagocytes are created equally: appreciating the heterogeneity of the hemophagocytic syndromes, Curr. Opin. Rheumatol. 24 (2012) 113.
[4] S. Zhang, Z. Wu, P. Li, Y. Bai, F. Zhang, Y. Li, Evaluation of the clinical performance of a novel chemiluminescent immunoassay for detection of anticardiolipin and anti-Beta2-glycoprotein 1 antibodies in the diagnosis of antiphospholipid syndrome, Medicine (Baltimore) 94 (2015) e2059.
[5] M. Arca, L. Fardet, L. Galicier, S. Riviere, C. Marzac, C. Aumont, et al., Prognostic factors of early death in a cohort of 162 adult haemophagocytic syndrome: impact of triggering disease and early treatment with etoposide, Br. J. Haematol. 168 (2015) 63.
[6] A.R. Han, H.R. Lee, B.B. Park, I.G. Hwang, S. Park, S.C. Lee, et al., Lymphoma-associated hemophagocytic syndrome: clinical features and treatment outcome, Ann. Hematol. 86 (2007) 493.
[7] L. Fardet, L. Galicier, O. Lambotte, C. Marzac, C. Aumont, D. Chahwan, et al., Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome, Arthritis Rheumatol. 66 (2014) 2613.
[8] C.E. Allen, X. Yu, C.A. Kozinetz, K.L. McClain, Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis, Pediatr. Blood Cancer 50 (2008) 1227.
[9] Y. Tang, X. Xu, H. Song, S. Yang, S. Shi, J. Wei, et al., Early diagnostic and prognostic significance of a specific Th1/Th2 cytokine pattern in children with haemophagocytic syndrome, Br. J. Haematol. 143 (2008) 84.
[10] S. Nanno, H. Koh, Y. Nakashima, T. Katayama, H. Okamura, S. Koh, et al., Diagnostic value of serum ferritin and the risk factors and cytokine profiles of hemophagocytic syndrome following allogeneic hematopoietic cell transplantation, Leuk. Lymphoma 58 (2017) 1664.
[11] H. Takada, Y. Takahata, A. Nomura, S. Ohga, Y. Mizuno, T. Hara, Increased serum levels of interferon-gamma-inducible protein 10 and monokine induced by gamma interferon in patients with haemophagocytic lymphohistiocytosis, Clin. Exp. Immunol. 133 (2003) 448.
[12] G.E. Janka, Familial and acquired hemophagocytic lymphohistiocytosis, Annu. Rev. Med. 63 (2012) 233.
[13] H.J. Chung, C.J. Park, J.H. Lim, S. Jang, H.S. Chi, H.J. Im, et al., Establishment of a reference interval for natural killer cell activity through flow cytometry and its clinical application in the diagnosis of hemophagocytic lymphohistiocytosis, Int. J. Lab. Hematol. 32 (2010) 239.
[14] S. Shabrish, M. Gupta, M. Madkaikar, A modified NK Cell degranulation assay applicable for routine evaluation of NK cell function, J. Immunol. Res. 2016 (2016) 3769590.
[15] C. Yang, X. Zhu, T. Zhang, Q. Ye, EBV-HLH children with reductions in CD4+ T cells and excessive activation of CD8+ T cells, Pediatr. Res. 82 (2017) 952.
[16] H. Hou, Y. Zhou, J. Yu, L. Mao, M.J. Bosco, J. Wang, et al., Establishment of the Reference Intervals of Lymphocyte Function in Healthy Adults Based on IFN-gamma Secretion Assay upon Phorbol-12-Myristate-13-Acetate/Ionomycin Stimulation, Front. Immunol. 9 (2018) 172.
[17] F. Wang, H. Hou, L. Xu, M. Jane, J. Peng, Y. Lu, et al., Tim-3 signaling pathway as a novel negative mediator in lipopolysaccharide-induced endotoxic shock, Hum. Immunol. 75 (2014) 470.
[18] S. Riviere, L. Galicier, P. Coppo, C. Marzac, C. Aumont, O. Lambotte, et al., Reactive hemophagocytic syndrome in adults: a retrospective analysis of 162 patients, Am. J. Med. 127 (2014) 1118.
[19] L. Han, L. Li, J. Wu, X. Li, L. Zhang, X. Wang, et al., Clinical features and treatment of natural killer/T cell lymphoma associated with hemophagocytic syndrome: comparison with other T cell lymphoma associated with hemophagocytic syndrome, Leuk. Lymphoma 55 (2014) 2048.
[20] J. Jia, Y. Song, N. Lin, W. Liu, L. Ping, W. Zheng, et al., Clinical features and survival of extranodal natural killer/T cell lymphoma with and without hemophagocytic syndrome, Ann. Hematol. 95 (2016) 2023.
[21] M. Xie, L. Li, L. Zhu, Yang X Zhou, Sun J An, et al., An effective diagnostic index for lymphoma-associated hemophagocytic syndrome, QJM (2018).
[22] N. Li, L. Zhang, J. Liu, J. Zhang, H.W. Weng, H.Y. Zhuo, et al., A clinical study of 21 patients with hemophagocytic syndrome in 295 cases diagnosed with nasal type, extranodal nature killer/T cell lymphoma, Cancer Biol. Ther. 18 (2017) 252.
[23] F. Li, P. Li, R. Zhang, G. Yang, D. Ji, X. Huang, et al., Identification of clinical features of lymphoma-associated hemophagocytic syndrome (LAHS): an analysis of 69 patients with hemophagocytic syndrome from a single-center in central region of China, Med. Oncol. 31 (2014) 902.
[24] I. Murohashi, K. Yoshida, N. Ihara, D. Wakao, F. Yagasaki, Y. Nakamura, et al., Serum levels of Thl/Th2 cytokines, angiogenic growth factors, and other prognostic factors in young adult patients with hemophagocytic syndrome, Lab. Hematol. 12 (2006) 71.
[25] L.T. My, B. Lien le, W.C. Hsieh, T. Imamura, T.N. Anh, P.N. Anh, et al., Comprehensive analyses and characterization of haemophagocytic lymphohistiocytosis in Vietnamese children, Br. J. Haematol. 148 (2010) 301.
[26] S. Ohga, A. Matsuzaki, M. Nishizaki, T. Nagashima, T. Kai, M. Suda, et al., Inflammatory cytokines in virus-associated hemophagocytic syndrome. Interferon-gamma as a sensitive indicator of disease activity, Am. J. Pediatr. Hematol. Oncol. 15 (1993) 291.
[27] H. Takada, S. Ohga, Y. Mizuno, A. Suminoe, A. Matsuzaki, K. Ihara, et al., Oversecretion of IL-18 in haemophagocytic lymphohistiocytosis: a novel marker of disease activity, Br. J. Haematol. 106 (1999) 182.
[28] K. Mazodier, V. Marin, D. Novick, C. Farnarier, S. Robitail, N. Schleinitz, et al., Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome, Blood 106 (2005) 3483.
[29] S. Mhatre, M. Madkaikar, K. Ghosh, M. Desai, V. Pujari, M. Gupta, Rapid flow cytometry based cytotoxicity assay for evaluation of NK cell function, Indian J. Exp. Biol. 52 (2014) 983.