Full-length Article Manuscript

Hemophagocytic Lymphohistiocytosis： A Skeleton in the Cupboard of Adult Idiopathic Inflammatory Myopathies

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Key Indexing Terms: hemophagocytic lymphohistiocytosis, dermatomyositis, polymyositis, interstitial lung disease, infection, outcome

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The sources of support: This study was supported in part by the grants from National Natural Science Foundation of China (81701600 and 81701602) and Medical Health Science and Technology Project of Zhejiang Provincial Health Commission (2018 KY067).

Conflict of interest: None declared.

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Short running head: Secondary hemophagocytic lymphohistiocytosis

Abstract

Objective: To clarify the prevalence, risk factors, outcome and outcome-related factors of hemophagocytic lymphohistiocytosis (HLH) in patients with dermatomyositis (DM), polymyositis (PM) or clinically amyopathic dermatomyositis (CADM).

Methods: Data of patients with DM, PM or CADM who were admitted to the First Affiliated Hospital of Zhejiang University (FAHZJU) from February 2011 to February 2019 were retrospectively collected. Patients diagnosed with HLH constituted the case group. A 1:4 case-control study was performed to identify risk factors for HLH in patients with DM, PM or CADM through comparison, univariate and multivariate logistic regression analysis. Intragroup comparison was made within HLH patients to identify factors influencing unfavorable short-term outcome.

Results: HLH was a rare (4.2%) but fatal (77.8%) complication in patients with DM, PM or CADM. The retrospective case-control study revealed that higher on-admission disease activity (P=0.008), acute exacerbation of interstitial lung disease (AE-ILD, P=0.002) and infection (P=0.002) were risk factors for complication of HLH in patients with DM, PM or CADM. The following intragroup comparison showed that higher on-admission disease activity (P=0.035) and diagnosis of CADM (P=0.039) might influence the short-term outcome of HLH patients. However, no risk factor was identified after false discovery rate correction.

Conclusion: In this study, secondary HLH was a fatal complication with higher on-admission disease activity, AE-ILD and infection working as risk factors. The underlying role of infection and autoimmune abnormality in HLH in connective tissue disease (CTD) was subsequently brought up. Clinical factors influencing the short-term outcome of patients with secondary HLH required further exploration.

Introduction

Autoimmune idiopathic inflammatory myopathy (IIM) encompasses a heterogeneous group of acquired skeletal muscle diseases, most of which share the clinical manifestation of relevant weakness owing to muscle inflammation and necrosis [1]. Dermatomyositis (DM) and polymyositis (PM) are two classical subtypes of IIM, whereas clinically amyopathic dermatomyositis (CADM) is a newly recognized subgroup of DM featuring typical skin rash of DM and slight muscular impairment[2,3]. Preceding studies have reported considerably high mortality rate in DM, PM or CADM patients. The 10-year survival rate for patients with DM, PM or CADM ranged from 51% to 91% in different researches [4]. An approximate in-hospital mortality rate of 4.5% was seen in two retrospective studies [4, 5]. In addition, extramuscular involvements, namely interstitial lung disease (ILD), arthritis, cardiac involvement and malignancies, add to the suffering and poor outcome of patients as well [3].

Previous studies also reported several haemophagocytic lymphohistiocytosis (HLH) cases in IIM patients. And the scarce cases of IIM patients with HLH were mostly categorized as DM. In addition to the primary form of the disease, HLH can occur secondary to malignancy, infection, autoimmune disease, or immune deficiency state caused by immunosuppressant therapy [6]. Unrestrained macrophage and T cell activation, cytokine storm, acquired defects in cytotoxicity, TLRs-related innate immune activation and defects in MAGT1, ITK, CD27, IKBKG, or GATA2 genes etc. were suggested to induce secondary HLH [7-10]. This life-threatening complication is uniformly fatal if not diagnosed in time and treated properly. However, knowledge on secondary HLH in patients with DM, PM or CADM was considerably limited. It is thus necessary to fill in the gaps by sorting out prevalence, risk factors and outcome for development of secondary HLH

in patients with DM, PM or CADM, and factors associated with outcome of these HLH patients.

In this study, we retrospectively reviewed the medical records of 424 patients with DM, PM or CADM who were admitted to our center from February 2011 to February 2019, and performed a case-control analysis to identify potential related risk factors for HLH among these patients. Subgroup analysis was as well made to preliminarily identify factors affecting short-term outcome of HLH patients.

Materials and methods

Patients

After acquiring the approval (Reference Number: 2019-646) of the Institutional Review Board (IRB) of the First Affiliated Hospital of Zhejiang University (FAHZJU) and obtaining written informed consent to utilize and publish clinical data from all patients involved, we retrieved medical records of adult patients who were hospitalized at FAHZJU with the diagnosis of DM, PM, or CADM from February 2011 to February 2019. The inclusion criteria of this study were: 1) age over 18 years old; 2) the diagnosis of DM or PM fulfilled the diagnostic criteria of Bohan and Peter [11], and the diagnosis of CADM met the criteria developed by Sontheimer and his colleagues [12]. Exclusion criteria were: 1) overlap syndromes with other connective tissue diseases (CTDs); 2) hospitalization for causes unrelated to myositis and its complications, such as fracture, pregnancy, cataract and appendicitis etc.; 3) loss to follow-up within 2 weeks after discharge.

Methods

Medical records of all patients included were retrospectively collected by reviewing the

electronic medical record (EMR) system. Data including demographic information, course of disease, duration of diagnosis delay, disease activity, clinical manifestations or complications, preceding comorbidities, harmful hobbies, imaging reports, laboratory findings and medications as well as short-term outcome were obtained and analyzed. On-admission disease activity was routinely assessed by the Myositis Disease Activity Assessment Visual Analogue Scales (MYOACT) within the first week of admission [13]. Diagnosis of HLH was based on the criteria proposed by the Histiocyte Society in 2004 (Supplementary table 1) [14], and was reevaluated by a hematologist afterwards. ILD and acute exacerbation of ILD (AE-ILD) were evaluated by radiologists using high-resolution computed tomography (HRCT). Diagnostic criteria for AE-ILD included previous or concurrent diagnosis of ILD, acute worsening or development of dyspnea typically <1 month duration, computed tomography with new bilateral ground-glass opacity and/or consolidation superimposed on a background pattern consistent or inconsistent with usual interstitial pneumonia pattern, and deterioration not fully explained by cardiac failure or fluid overload [15,16]. Diagnosis of bacterial, fungal or tuberculosis infection was a comprehensive decision based on the essential microbiological findings of sputum or blood, together with clinical manifestations, radiographic and laboratory abnormalities. Meanwhile diagnosis of virus infection, to be specific, Epstein-Barr virus (EBV) and Cytomegalo virus infection (CMV), relied on the screening of serum antibody and DNA of these two virus. A combined positive result of serum IgM and DNA of EBV or CMV made the diagnosis solid. Identification of gastrointestinal hemorrhage was based on repeated positive results of fecal occult blood test. Regarding antibacterial or antifungal therapies, antibiotics based on drug resistant testing (DST-based antibiotics), use of third-line antibiotics and intravenous antifungal therapy were regarded as active and potent treatment for infection [17-19].

Of which, third-line antibiotics referred to carbapenem, vancomycin, linezolid [19]. Prophylactic application of sulfamethoxazole (SMZ) was a measure to prevent fungal infection when using steroids and other immunosuppressive therapies. Short-term mortality, or unfavorable short-term outcome, referred to in-hospital mortality or death within 2 weeks of hospital discharge.

To probe into factors exerting significant influence on development of HLH within patients with DM, PM or CADM, a case-control study was performed. In-hospital adult patients with DM, PM or CADM, who satisfied the inclusion/exclusion criteria and was diagnosed with HLH, constituted the case group. To construct the control group, adult patients without HLH, who were admitted to the in-patient department of FAHZJU from the outpatient department and emergency room, satisfied the diagnostic criteria of DM, PM or CADM and other inclusion/exclusion criteria, were selected using a systematic sampling method by matching age and sex with cases with HLH at a proportion of 1:4. Comparisons and logistic regression analysis were performed between the two groups. To clarify the time axis of risk factors and result, only clinical manifestations or complications that happened before the diagnosis of HLH would be taken into account for HLH patients. In order to identify potential factors affecting the short-term outcome of the HLH patients involved, the identified HLH patients were further divided into two groups: patients who died in hospital or within 2 weeks of hospital discharge were categorized as the mortality group, and those who survived after 2 weeks of hospital discharge were defined as the survival group. Afterwards comparisons were made between the two subgroups.

Statistical analysis

Statistical analysis was performed using SPSS 22.0 (Chicago, IL, USA) and R 3.6.1. The normality of continuous variables was tested by the Kolmogorov-Smirnov goodness-of-fit model.

In studied variables, continuous variables were expressed as mean ± SD if normally distributed and median (quartiles) if skewed. Ordinal categorical variables were as well shown as median (quartiles). Unordered categorical variables were presented as numbers and percentages. Independent sample t-test was used to compare normally distributed continuous variables. And Mann-Whitney U test was applied to compare skewed continuous variables or ordinal categorical variables. Chi-square test and Fisher's exact test were used to compare unordered categorical variables. P values in comparisons were adjusted by false discovery rate (FDR) correction, using p.adjust function in R.3.6.1, to obtain adjusted p values. All tests were two-sided, and a p-adjusted<0.05 was considered statistically significant. Univariate and multivariate logistic regression analyses were subsequently adopted to uncover risk factors for HLH in patients with PM, DM or CADM. Explanatory factors with P value<0.05 in the univariate logistic regression analysis were entered into the multivariate logistic regression analysis. Results from the multivariate logistic regression analysis were presented as an odds ratio (OR) with 95% confidence interval (CI). A two-sided P value less than 0.01 was considered to be statistically significant.

Results

A total of 424 patients treated at FAHZJU with a diagnosis of DM, PM or CADM between February 2011 and February 2019 were included, encompassing 213 with DM, 170 with PM and 41 with CADM. 18 out of 424 patients were diagnosed with HLH during their stay in hospital, and all of which can be categorized as secondary HLH (Table 1). The incidence of secondary HLH was 4.2%. The average age in the case group was 58.1±14.4 years, which was significantly higher than

that of the non-HLH patients (50.4±14.1 years, P =0.007). Among the 18 HLH patients, 5 were males and 13 were females. The proportion of males in HLH patients was not significantly different from that in non-HLH patients (27.8% vs. 29.8%, P = 0.854). Short-term mortality rate for HLH and non-HLH patients were 77.8% vs 6.5% (P<0.001).

In total, 18 HLH patients and 72 non-HLH patients were included in the case-control analysis to identify risk factors for HLH in patients with DM, PM or CADM. On-admission disease activity, which was evaluated by MYOACT score, was significantly higher in patients complicated with HLH (P<0.001). Besides, the case group presented more frequently with AE-ILD (P<0.001), gastrointestinal hemorrhage (P=0.007), infection (P<0.001), hepatic insufficiency (P=0.039), on-admission hyperferritinemia (p=0.001), hypertension (P=0.002). Mycophenolate mofetil (MMF, P=0.007) was more often used in patients who did not suffer from secondary HLH. However, after FDR correction, only on-admission disease activity (P-adjusted<0.001), AE-ILD (P-adjusted<0.001), infection (P-adjusted<0.001), on-admission hyperferritinemia (P-adjusted=0.015) and hypertension (P-adjusted=0.024) remained significantly different. (Table 2)

Univariate analysis showed that there were 9 factors associated with HLH at the level of P< 0.05. These factors included on-admission disease activity (P=0.001), AE-ILD (P<0.001), gastrointestinal hemorrhage (P=0.005), infection (P<0.001), hepatic insufficiency (P=0.049), on-admission hyperferritinemia (p=0.009), hypertension (P=0.004), MMF (P=0.024) and immunoglobulin (P=0.045). The multivariate logistic regression analysis then uncovered the skeletons in the HLH cupboard. Higher on-admission disease activity (P=0.008), AE-ILD (P=0.002) and infection (P=0.002) were recognized as risk factors for complication of HLH in patients with DM, PM or CADM. (Table 3, Supplementary table 2)

14 out of 18 HLH patients died in hospital or within 2 weeks of hospital discharge. In addition to 11 HLH patients with DM, we also found 5 PM patients and 2 CADM patients who as well suffered from HLH. Infection happened to 15 out of 18 adult HLH patients (Table 4, Supplementary data 1). Bacterial (9 cases, 50%) and fungal (7 cases, 38.9%) infection were recognized as the two most common infections in HLH patients. 2 patients were found to complicate with solid tumors (breast cancer and adenocarcinoma of thyroid). Similar results were seen in patients of the control group with merely 1 hematologic malignancies in 8 cases complicated with carcinoma. Based on repeated positive results of fecal occult blood test, gastrointestinal hemorrhage was seen in 7 patients. Hemophagocytosis was observed in bone marrow smear (BMS) of 9 patients (Supplementary figure 1). And AE-ILD was found in 13 patients (Figure 1). Due to the limited number of HLH cases, only Fisher's exact test and Mann-Whitney U test were performed in the process of identifying potential factors influencing short-term outcome of patients with HLH. On-admission disease activity (P=0.035), which was evaluated by MYOACT score, was as well higher for patients in the mortality group. And HLH patients with the diagnosis of CADM (P=0.039) were found to have more favorable short-term outcome, After FDR correction, nevertheless, no factor was found to be significantly correlated with the short-term outcome of patients with secondary HLH. (Table 5)

Discussion

To date, this is the first study to probe into the risk factors for HLH in IIM patients, and potential factors affecting the short-term outcome of these patients. In terms of HLH in autoimmune diseases, previous studies revealed that HLH was more commonly seen in systemic juvenile idiopathic arthritis (sJIA), systemic lupus erythematosus (SLE), adult onset still disease (AOSD) and Kawasaki

disease [20-23]. In this study, the incidence rate of HLH in patents with DM, PM or CADM was 4.2%. Rare as it was, the short-term mortality rate in patients with HLH was significantly higher than that in non-HLH patients (77.8% vs 6.5%, P<0.001), which made it essential to identify risk factors for HLH and potential factors influencing the outcome of patients complicated with HLH.

Patients with DM, PM or CADM regularly receive immunosuppressive treatment. The immunosuppressive state, together with the pathogenesis of idiopathic inflammatory myopathy, leaves the patients vulnerable to malignancy and infection. Preceding study indicated that malignancy was the most common trigger for development of secondary HLH in adult patients [23]. Hematologic malignancies such as lymphoma and leukemia accounted for most malignancy-related HLH. Meanwhile the association with solid tumor was rare [8]. However, malignancy was not found to be significantly related to HLH in this study, which might be contributed to the high proportion of solid tumors in patients with DM, PM or CADM.

In addition, hyperferritinemia was found to be significantly related to development of HLH in comparison. Preceding studies revealed that overexpression of ferritin not only worked as a consequence of inflammation, but also promoted the development of secondary HLH by perpetuating production of cytokines, recruitment and proliferation of macrophages via a vicious loop [24,25]. Besides, overexpression of ferritin could also be induced by activity of autoimmune diseases and infection, which, to some extent, explained the absence of significance after adjusting for factors including infection and disease activity. Nevertheless, the potential role of ferritin in development of secondary HLH demands more attention and further researches.

On the other hand, infection happened to 15 (83.3%) out of the 18 HLH patients in this study, and was proved to be risk factor for HLH in patients with DM, PM or CADM. Multiple infectious

agents including virus, bacteria, fungus and parasite have been identified to induce secondary HLH in patients with various diseases. And infection accounted for approximately 30% of all-cause secondary HLH in multiple studies [26-28]. However, in a Spanish study centered on patients receiving potent immunosuppressive treatment, 2/3 of secondary HLH patients had suspected infectious triggers [29]. Immunosuppressive medications were hereby reckoned to contribute to higher incidence of infection in this study. There existed multiple hypotheses for mechanism of infection-related HLH. Suspected mechanisms included uncontrolled macrophage and T cell activation, acquired defects in cytotoxicity, cytokine storm and TLRs-related innate immune activation. Exuberant cytokine release was commonly seen in infection [30], and was hypothesized to impair NK cell cytotoxicity. In addition, several infectious agents were capable of suppressing CTL and NK cell cytotoxicity directly [26]. The deficiency in cytolytic activity resulted in persistent activation of lymphocytes and histiocytes, which might in turn promote secretion of pro-inflammatory cytokines [31]. A few TLRs had also been identified in human or mice to respond to microbial antagonists and subsequently drove innate immune response [32].

Preceding study found that elevated systemic evaluating score was correlated with development of secondary HLH, or macrophage activation syndrome, in patients with adult onset still disease [33]. For patients with DM, PM or CADM, higher on-admission disease activity, which was evaluated by MYOACT score, was also found to be a significant risk factor for development of HLH in these patients. The systemic MYOACT score and the multiple muscle strength assessments all belong to the core set measures for IIM [34]. The MYOACT score was a subjective and rough evaluation consisting of a series of 10 cm visual analogue scales concerning different organs or systems [35]. Meanwhile muscle strength assessments were usually more

detailed and objective in evaluating muscular involvement. Faced with the heterogeneity of muscular involvement of DM, PM and CADM, however, the undivided muscle strength assessments failed to reflect the disease activity or damage in IIM patients. And creatine kinase was not an appropriate evaluating tools for similar consideration. Under this circumstance, the integrity of MYOACT score would alleviate heterogeneity of different subtypes and reflected the overall disease activity. In addition, most muscle strength assessments shared defects including poor sensitivity to change as well as floor and ceiling effects in natural history studies [34]. And some assessments like manual muscle testing could not fully evaluate muscle impairment by assessing only the strength, not the endurance of the muscle [35]. Besides, MYOACT score was also found to be consistent with the inflammatory level of IIM patients and would hereby reflect the autoimmune abnormality in IIM [36-38]. The correlation between disease activity and secondary HLH also indicated the potential role of autoimmune abnormality in development of HLH in IIM patients.

In multivariate logistic regression analysis, AE-ILD remained a significant risk factor for HLH in patients with DM, PM or CADM after adjusting for factors including infection and immunosuppressant therapies. And this is the first study to identify exacerbation of ILD as risk factor for secondary HLH. The relevance between AE-ILD and development of HLH might reside in their overlapped pathological mechanisms. Macrophage activation and exuberant release of cytokines were speculated to promote secondary HLH. Several studies had observed significant elevation of cytokines including IL-6 and IL-8, and M2 macrophage activation in patients with ILD exacerbation, and it was found to be associated with worse outcome [39-43]. The underlying overlapped pathological mechanism linked exacerbation of ILD with development of HLH. After

adjusting for factors including infections and immunosuppressant medications, an acute exacerbation of connective-tissue-disease-related ILD (CTD-ILD) might, to some extent, be viewed as a reflection of aggravation of autoimmune abnormality, In this case, AE-ILD indicated an underlying deterioration of autoimmune abnormality in development of HLH in the setting of CTD.

Comorbid illnesses including infection were found to play a contributing role in unfavorable outcome of patients with secondary HLH [28, 44]. Although patients with bacterial infection all ended up in unfavorable outcome, no significant correlation was identified between infectious agents and short-term outcome possibly owing to the small sample size in this study. And secondary HLH patients with CADM seemed to have more favorable outcome compared with other IIM subtypes, which could be seen as an accidental event due to the small sample size, or a result of unknown distinctive pathogenesis in CADM. In addition, higher level of on-admission disease activity, which was evaluated by MYOACT score and reflected severity of IIM, was also found in HLH patients with unfavorable short-term outcome. Despite the negative result after FDR correction, the roles of on-admission disease activity, bacterial infection and diagnosis of CADM deserve further verification in larger cohort.

The high mortality rate of patients complicated with HLH (77.8%) also precipitated review of therapeutic strategy. Frequent complication of infections facilitated use of antibiotics and antifungal therapy, and prevented us from using stronger chemotherapy or immunotherapy in clinical practice. Application of MMF was not found to improve the outcome of HLH patients partly due to its scarce application in secondary HLH patients, whereas it significantly reduced the incidence of secondary HLH in comparison. MMF reversibly inhibits inosine monophosphate dehydrogenase, leading to decreased B cell and T cell proliferation and decreased cytokine

production, which might consequently suppress the dysfunction in secondary HLH [45].In addition, two SLE patients with secondary HLH was reported to be successfully treated by MMF, indicating the potential of MMF in curing secondary HLH in CTD patients [46, 47]. The significance, however, vanished after adjusting for factors including infection, disease activity etc. The unclarified but possible role of MMF in preventing and treating secondary HLH hence demanded further exploration in larger cohort. Besides, DST-based antibiotics (P=0.074, Table 2) tended to reduce the incidence of secondary HLH in the setting of bacterial infection, indicating the role of standardized and potent anti-bacterial treatment in prevention of secondary HLH. Despite the active treatment targeting infection, the outcome of HLH patients was far from optimistic. Treatment of secondary HLH depends on the suppression of hyperinflammation and targeting of the underlying disease. Application of stronger chemotherapy or immunotherapy, which was based on the HLH-94 and HLH-2004 protocols [14, 48], was hereby deemed essential in spite of the frequent complication of infection. However, Kumar etc. questioned the effect of HLH-2004 in secondary HLH and proposed a modified therapeutic approach including anakinra, IVIG, steroids, cyclosporine and tocilizumab [49]. Continuous studies on pathogenesis of secondary HLH also suggested potential effect of therapies targeting IFN-γ, CD52 and Janus kinase pathways, etc. [50]

The most significant limitations of this study were the retrospective and observational nature of the study and the small sample size. Two parameters in the diagnostic criteria of HLH, namely soluble CD25 and NK cell activity, were not measured due to their unavailability. The incidence of secondary HLH in this study might hereby be underestimated. Several myositis antibodies, namely anti-melanoma differentiation-associated gene 5 antibody etc., were not included in the

study due to their absence in over 2/3 of patients involved and the retrospective nature of the study. Last but not least, we failed to provide valid suggestions on treatment of secondary HLH in IIM patients due to the serious heterogeneity of therapeutic regimens and the rarity of secondary HLH in these patients. In spite of all the limitations, we intended to shed some light on the future study of secondary HLH in IIM patients.

Conclusion

In IIM patients, secondary HLH was a fatal complication with higher on-admission disease activity, AE-ILD and infection as risk factors, which indicated the potential role of infection and autoimmune abnormality in development of HLH in the setting of CTD. In addition, the role of higher on-admission disease activity, bacterial infection and diagnosis of CADM etc. in the short-term outcome of patients with secondary HLH required further exploration.

Aknowledgement

The authors appreciate Yile Zhou's assistance in verification of HLH, and the help of Bei Xu, Yuli Wang in diagnosis of ILD and AE-ILD.

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Figure 1: AE-ILD of a HLH patient in this study (a/b: before exacerbation, c/d: after exacerbation).

AE-ILD: Acute exacerbation of intersstitial lung disease; HLH: Hemophagocytic lymphohistiocytosis.

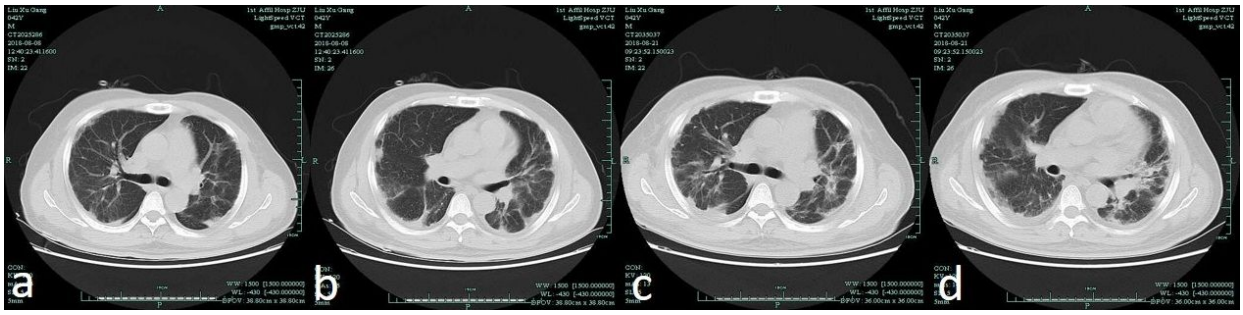

Table 1: Overview of clinical features included in revised diagnostic guideline of HLH within case group and control group.

HLH: HLH: Hemophagocytic lymphohistiocytosis; NK cell: Natural killer cell; IL-2: Interleukin-2.

Clinical Features	Case Group(18)	Control Group(72)
A molecular diagnosis consistent with HLH	**NA**	**NA**
Fever (≥38.5°C for ≥7 days)	**18(100.0%)**	**11(15.3%)**
Splenomegaly	**13(72.2%)**	**6(8.3%)**
Cytopenias (affecting 2 of 3 lineages in the peripheral blood)	**18(100.0%)**	**0(0.0%)**
Hemoglobin <90 g/L	17(94.4%)	5(6.9%)
Platelets <100*10^9/L	18(100.0%)	8(11.1%)
Neutrophils <1.0*10^9/L	5(27.8%)	1(1.4%)
Hypertriglyceridemia and/or hypofibrinogenemia	**18(100.0%)**	**14(19.4%)**
Fasting triglycerides≥3.0 mmol/L (i.e., 265 mg/dl)	10(55.6%)	11(15.3%)
Fibrinogen≤1.5 g/L	12(66.7%)	3(4.2%)
Hemophagocytosis in bone marrow	**9 (50.0%)**	**0(0.0%)**
Low or absent NK cell activity	**NA**	**NA**
Ferritin≥500 mg/L	**18(100.0%)**	**40(55.6%)**
Soluble CD25 (i.e., soluble IL-2 receptor)≥2,400 U/ml	**NA**	**NA**

Table 2: Comparison of clinical characteristics between case group and control group.

HLH: hemophagocytic lymphohistiocytosis; P-adjusted: adjusted p value after false discovery rate (FDR) correction; y: years; m: months; ILD: Interstitial lung disease; AE-ILD: Acute exacerbation of interstitial lung disease; CK: Creatine kinase; LDH: Lactate dehydrogenase; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; On-admission hyperferritinemia: on-adimission serum ferritin≥500ng/ml; ANA: Antinuclear antibody; ACA: Anti-centromere antibody; MMF: Mycophenolate mofetil; DMARDs: disease-modifying anti-rheumatic drugs; DST-based antibiotics: antibiotics based on drug resistant testing; Prophylactic SMZ: Prophylactic application of sulfamethoxazole; DM: dermatomyositis; PM: Polymyositis; CADM: Clinically amyopathic dermatomyositis.

Factors	HLH(18)	Non-HLH(72)	P value	P-adjusted
Age(y)	58.1±14.4	58.1±13.0	0.991	1.000
Sex(male/female)	5/13	20/52	1.000	1.000
Course of disease(m)	3.0(1.0, 8.5)	3.0(1.0, 6.0)	0.857	1.000
Duration of diagnosis delay(m)	2.0(1.0, 3.5)	2.0(1.0,4.8)	0.815	1.000
Disease activity				
On-admission disease activity	12.0(9.0, 15.3)	9.0(6.0, 10.8)	<0.001	<0.001
Clinical manifestations or complications				
Heliotrope rash	9(50.0%)	30(41.7%)	0.523	1.000
Gottron's sign	9(50.0%)	30(41.7%)	0.523	1.000
Periungual erythema	2(11.1%)	9(12.5%)	1.000	1.000
Mechanic's hands	1(5.6%)	10(13.9%)	0.573	1.000
Raynaud's phenomenon	0(0.0%)	7(9.7%)	0.376	0.893
Muscle pain	9(50%)	28(38.9%)	0.391	0.893
Muscle weekness	16(88.9%)	55(76.4%)	0.401	0.893
Joint pain	5(27.8%)	17(23.6%)	0.951	1.000
Joint swelling	3(16.7%)	12(16.7%)	1.000	1.000
Dysphagia	7(38.9%)	17(23.6%)	0.311	0.862
Dysarthria	1(5.6%)	5(6.9%)	1.000	1.000
Respiratory muscle involvement	0(0.0%)	3(4.2%)	1.000	1.000
ILD	18(100.0%)	63(87.5%)	0.253	0.793
AE-ILD	13(72.2%)	14(19.4%)	<0.001	<0.001
Gastrointestinal hemorrhage	7(38.9%)	7(9.7%)	0.007	0.061
Cardiac involvement	3(16.7%)	6(8.3%)	0.539	1.000

Infection	15(83.3%)	21(29.2%)	<0.001	<0.001
Carcinoma	2(11.1%)	8(11.1%)	1.000	1.000
On-admission laboratory findings				
CK(U/L)	185.5(56.5,1401.8)	752.0(109.8,3498.8)	0.101	0.423
LDH(U/L)	584.5(356.0,733.8)	500.0(345.0,782.8)	0.709	1.000
ESR(mm/h)	30.5(8.0,40.3)	20.0(10.3,34.0)	0.548	1.000
CRP(mg/L)	8.4(2.8,60.2)	8.5 (3.0,24.9)	0.614	1.000
Hepatic insufficiency	15(83.3%)	41(56.9%)	0.039	0.297
Renal insufficiency	4(22.2%)	6(8.3%)	0.208	0.746
On-admission hyperferritinemia	17(94.4%)	37(51.4%)	0.001	0.015
ANA	9(50.0%)	51(70.8%)	0.094	0.423
ANA titer	10.0(0.0,25.0)	20.0(0.0,80.0)	0.059	0.400
Anti-SSA	2(11.1%)	13(18.1%)	0.724	1.000
Anti-SSA52	6(33.3%)	32(44.4%)	0.393	0.893
Anti-SSB	0(0.0%)	2(2.8%)	1.000	1.000
Anti-Ro52	0(0.0%)	0(0.0%)	NA	NA
Anti-RNP	0(0.0%)	5(6.9%)	0.579	1.000
Anti-Jo-1	1(5.6%)	6(8.3%)	1.000	1.000
ACA	0(0.0%)	2(2.8%)	1.000	1.000
Comorbidities/Harmful hobbies				
Smoking	2(11.1%)	9(12.5%)	1.000	1.000
Alcohol abuse	1(5.6%)	12(16.7%)	0.410	0.893
Hypertension	11(61.1%)	17(23.6%)	0.002	0.024
Diabetes	2(11.1%)	8(11.1%)	1.000	1.000
Allergy	1(5.6%)	14(19.4%)	0.289	0.839
Medications				
Steroid	18(100.0%)	72(100.0%)	NA	NA
MMF	1(5.6%)	28(38.9%)	0.007	0.061
Thalidomide	2(11.1%)	11(15.3%)	0.940	1.000
Hydroxychloroquine	3(16.7%)	12(16.7%)	1.000	1.000
Cyclosporine	1(5.6%)	1(1.4%)	0.362	0.893
Azathioprine	0(0.0%)	0(0.0%)	NA	NA
Methotrexate	2(11.1%)	2(2.8%)	0.177	0.675
Immunoglobulin	7(38.9%)	12(16.7%)	0.081	0.412
Cyclophosphamide	0(0.0%)	1(1.4%)	1.000	1.000
Steroid monotherapy	6(33.3%)	23(31.9%)	0.910	1.000
Steroid+DMARDs	5(27.8%)	37(51.4%)	0.073	0.410
Steroid+immunoglobulin	5(27.8%)	7(9.7%)	0.104	0.423
Steroid+DMARDs+immunoglobulin	2(11.1%)	5(6.9%)	0.922	1.000
DST-based antibiotics	6/9(66.7%)	11/11(100%)	0.074	0.410

Third-line antibiotics	**6/9(66.7%)**	**7/11(63.6%)**	**1.000**	**1.000**
Intravenous antifungal drugs	**7/7(100.0%)**	**9/13(69.2%)**	**0.249**	**0.793**
Prophylactic SMZ	**2(11.1%)**	**3(4.2%)**	**0.260**	**0.793**
IIM subtypes				
DM	**11(61.1%)**	**43(59.7%)**	**0.914**	**1.000**
PM	**5(27.8%)**	**25(34.7%)**	**0.576**	**1.000**
CADM	**2(11.1%)**	**4(5.6%)**	**0.595**	**1.000**

Table 3: Multivariate logistic regression analysis of risk factors for HLH in patients with DM, PM or CADM.

HLH: hemophagocytic lymphohistiocytosis; DM: dermatomyositis; PM: Polymyositis; CADM: Clinically amyopathic dermatomyositis; OR value: Odds ratio value; 95%Cl: 95% Confidence interval; AE-ILD: Acute exacerbation of interstitial lung disease.

Factors	P value	OR value	95%Cl
On-admission disease activity	**0.008**	**1.355**	**1.083~1.694**
AE-ILD	**0.002**	**10.192**	**2.329~44.601**
Infection	**0.002**	**13.169**	**2.568~67.537**

Table 4: Overview of infection subtypes within case group and control group.

Infection subtypes	Case Groups(18)	Control groups(72)
Bacterial infection	9(50.0%)	11(15.3%)
Fungal infection	7(38.9%)	13(18.1%)
Tuberculosis infection	1(5.6%)	1(1.4%)
Virus infection	1(5.6%)	1(1.4%)

Table 5: Comparison of clinical characteristics between mortality group and survival group.

P-adjusted: adjusted p value after false discovery rate (FDR) correction; ILD: Interstitial lung disease; AE-ILD: Acute exacerbation of interstitial lung disease; MMF: Mycophenolate mofetil; DMARDs: disease-modifying anti-rheumatic drugs; DST-based antibiotics: antibiotics based on drug resistant testing; Prophylactic SMZ: Prophylactic application of sulfamethoxazole; DM: dermatomyositis; PM: Polymyositis; CADM: Clinically amyopathic dermatomyositis.

Factors	Mortality group(14)	Survival group(4)	P value	P-adjusted
Age(y)	62.0(42.0,66.5)	66.0(64.0,72.5)	0.101	0.612
Sex(Male/Female)	4/10	1/3	1.000	1.000
Course of disease(m)	4.0(2.0,16.8)	1.0(1.0,4.0)	0.101	0.612
Duration of diagnosis delay(m)	2.5(2.0,3.8)	1.0(1.0,4.0)	0.192	0.864
Disease activity				
On-admission disease activity	13.0(9.8,16.0)	9.0(6.8,10.5)	0.035	0.612
Clinical features				
Fever	14(100.0%)	4(100.0%)	NA	NA
Lymphadenectasis	6(42.9%)	3(75.0%)	0.576	1.000
Splenomegaly	10(71.4%)	3(75.0%)	1.000	1.000
Hepatomegaly	2(14.3%)	0(0.0%)	1.000	1.000
Hyperferritinemia (Ferritin≥500ng/ml)	14(100.0%)	4(100.0%)	NA	NA
Neutropenia (Neutrophil count<1.0*10^9/L)	4(28.6%)	1(25.0%)	1.000	1.000
Anemia (Hemoglobin<90g/L)	14(100.0%)	3(75.0%)	0.222	0.888
Thrombocytopenia (Platelet count<100*10^9/L)	14(100.0%)	4(100.0%)	NA	NA
Hypofibrinogenemia (fibrinogen ≤1.5g/L)	10(71.4%)	2(50.0%)	0.569	1.000
Hypertriglyceridemia (Triglyceride≥3.0 mmol/L)	7(50.0%)	3(75.0%)	0.588	1.000
Bone marrow involvement (Hemophagocytosis in bone marrow)	8(57.1%)	1(25.0%)	0.576	1.000

ILD	14(100.0%)	4(100.0%)	NA	NA
AE-ILD	10(71.4%)	3(75.0%)	1.000	1.000
Gastrointestinal hemorrhage	7(50.0%)	0(0.0%)	0.119	0.612
Cardiac involvement	3(21.4%)	0(0.0%)	1.000	1.000
Carcinoma	1(7.1%)	1(25.0%)	0.405	1.000
Infection	13(92.9%)	2(50.0%)	0.108	0.612
Bacterial infection	9(64.3%)	0(0.0%)	0.082	0.612
Fungal infection	5(35.7%)	2(50.0%)	1.000	1.000
Tuberculosis infection	1(7.1%)	0(0.0%)	1.000	1.000
Virus infection	1(7.1%)	0(0.0%)	1.000	1.000
Medications				
Steroids	14(100.0%)	4(100.0%)	NA	NA
MMF	1(7.1%)	0(0.0%)	1.000	1.000
Thalidomide	2(14.3%)	0(0.0%)	1.000	1.000
Hydroxychloroquine	2(14.3%)	1(25.0%)	1.000	1.000
Cyclosporine	1(7.1%)	0(0.0%)	1.000	1.000
Azathioprine	0(0.0%)	0(0.0%)	NA	NA
Methotrexate	1(7.1%)	1(25.0%)	0.405	1.000
Immunoglobulin	6(42.9%)	1(25.0%)	1.000	1.000
Cyclophosphamide	0(0.0%)	0(0.0%)	NA	NA
Steroid monotherapy	5(35.7%)	1(25.0%)	1.000	1.000
Steroid+DMARDs	3(21.4%)	2(50.0%)	0.533	1.000
Steroid+immunoglobulin	4(28.6%)	1(25.0%)	1.000	1.000
Steroid+DMARDs+immunoglobulin	2(14.3%)	0(0.0%)	1.000	1.000
DST-based antibiotics	6/9(66.7%)	0/0	NA	NA
Third-line antibiotics	6/9(66.7%)	0/0	NA	NA
Intravenous antifungal drugs	5/5(100%)	2/2(100%)	NA	NA
Prophylactic SMZ	1(7.1%)	1(25.0%)	0.405	1.000
IIM subtypes				
DM	9(64.3%)	2(50.0%)	1.000	1.000
PM	5(35.7%)	0(0.0%)	0.278	1.000
CADM	0(0.0%)	2(50.0%)	0.039	0.612