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Hemophagocytic Lymphohistiocytosis: Prevalence, Risk Factors, Outcome, and Outcome-related Factors in Adult Idiopathic Inflammatory Myopathies

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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 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 among patients with HLH 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 patients with HLH. 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 was subsequently noted. Clinical factors influencing the short-term outcome of patients with secondary HLH require further study.

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

Autoimmune idiopathic inflammatory myopathy (IIM) encompasses a heterogeneous group of acquired skeletal muscle diseases, most of which share the clinical manifestation of weakness due to muscle inflammation and necrosis[1]. Dermatomyositis (DM) and polymyositis (PM) are 2 classic 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 rates in patients with DM, PM, or CADM. The 10-year survival rate for patients with DM, PM, or CADM ranged from 51% to 91% in different studies[4]. An approximate in-hospital mortality rate of 4.5% was seen in 2 retrospective studies[4,5]. Extramuscular conditions such as interstitial lung disease (ILD), arthritis, cardiac involvement, and malignancies add to the burden and poor outcome of patients[3].

Previous studies also reported several hemophagocytic lymphohistiocytosis (HLH) cases in patients with IIM. And the few cases of IIM 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]. These were suggested to induce secondary HLH: unrestrained macrophage and T cell activation, cytokine storm, acquired defects in cytotoxicity, Toll-like receptors (TLR)–related innate immune activation, and defects in *MAGT1, ITK, CD27, IKBKG,* or *GATA2* genes, among other things[7,8,9,10]. This life-threatening complication is uniformly fatal if not diagnosed in time and treated properly. However, knowledge about 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.

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In our 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 done to tentatively identify factors affecting short-term outcomes of patients with HLH.

MATERIALS AND METHODS

Patients. The Institutional Review Board of the First Affiliated Hospital of Zhejiang University (FAHZJU) gave approval for the study (Reference Number: 2019-646), and written informed consent was obtained from all patients to use and publish clinical data. 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; (2) the diagnosis of DM or PM fulfilled the diagnostic criteria of Bohan and Peter[11], or the diagnosis of CADM met the criteria developed by Sontheimer[12]. Exclusion criteria were (1) overlap syndromes with other connective tissue diseases (CTD); (2) hospitalization for causes unrelated to myositis and its complications, such as fracture, pregnancy, cataract, appendicitis; and (3) loss to followup within 2 weeks after discharge.

Methods. Medical records of all patients included were retrospectively collected by reviewing the electronic medical record system. Data were obtained and analyzed, including demographic information, course of disease, duration of diagnosis delay, disease activity, clinical manifestations or complications, preceding comorbidities, harmful habits, imaging reports, laboratory findings, and medications as well as short-term outcome. 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, available with the online version of this article)[14], and was reevaluated by a hematologist afterward. ILD and acute exacerbation of ILD (AE-ILD) were evaluated by radiologists using high-resolution computed tomography. 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 viral infection [i.e., Epstein-Barr virus (EBV) and cytomegalovirus (CMV) infection] relied on the screening of serum antibody and DNA of these 2 viruses. A combined positive result of serum IgM and DNA of EBV or CMV confirmed the diagnosis. Identification of gastrointestinal (GI) 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), third-line antibiotics, and intravenous antifungal therapy were regarded as active and potent treatment for infection[17,18,19]. Third-line antibiotics referred to carbapenem, vancomycin, and linezolid[19]. Prophylactic application of sulfamethoxazole 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.

A case-control study was performed to probe into factors exerting significant influence on development of HLH in patients with DM, PM, or CADM. In-hospital adult patients with DM, PM, or CADM who satisfied the inclusion/exclusion criteria and were diagnosed with HLH constituted the case group. For the control group, adult patients without HLH who were admitted to the in-patient department of FAHZJU from the outpatient department and emergency room and who 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 2 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 patients with HLH. To identify potential factors affecting the short-term outcome of the HLH patients involved, the patients were further divided into 2 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. Afterward, comparisons were made between the 2 subgroups.

Statistical analysis. Statistical analysis was performed using SPSS 22.0 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 also 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. The 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 2-sided, and a p-adjusted value < 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 OR with 95% CI. A 2-sided unadjusted p value < 0.01 was considered 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. Eighteen out of 424 patients were diagnosed with HLH during their hospital stay; all cases were 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 older than among the 406 non-HLH patients (50.4 ± 14.1 yrs, p = 0.007). Among the 18 HLH patients, 5 were men and 13 were women. The proportion of males among patients with HLH was not significantly different from that in non-HLH patients (27.8% vs 29.8%, p = 0.854). Short-term mortality rates for HLH and non-HLH patients were 77.8% versus 6.5% (p < 0.001).

There were 18 HLH patients and 72 matched non-HLH patients 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). In addition, the case group presented

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

Clinical Features	Case Group, n = 18	Control Group, n = 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) ≥ 2400 u/ml	NA	NA

Data are n (%). HLH: Hemophagocytic lymphohistiocytosis; NK cell: natural killer cell; IL-2: interleukin 2; NA: not applicable.

more frequently with AE-ILD (p < 0.001), GI hemorrhage (p = 0.007), infection (p < 0.001), hepatic insufficiency (p = 0.039), on-admission hyperferritinemia (p = 0.001), and hypertension (HTN; p = 0.002). Mycophenolate mofetil (MMF; p = 0.007) was more often used in patients who did not have secondary HLH. However, after FDR correction, only these factors remained significantly different: 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 HTN (p-adjusted = 0.024; 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), GI hemorrhage (p = 0.005), infection (p < 0.001), hepatic insufficiency (p = 0.049), on-admission hyperferritinemia (p = 0.009), HTN (p = 0.004), MMF (p = 0.024), and immunoglobulin (p = 0.045). The multivariate logistic regression analysis then revealed some unexpected findings about HLH. 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, and Supplementary Table 2, available with the online version of this article).

Fourteen out of 18 patients with HLH died in hospital or within 2 weeks of hospital discharge. In addition to 11 HLH patients with DM, we also found 5 patients with PM and 2 patients with CADM who had HLH. Infection occurred in 15 out of 18 adult cases of HLH (Table 4, and Supplementary Data 1, available with the online version of this article). Bacterial (9 cases, 50%) and fungal (7 cases, 38.9%) infection were recognized as the 2 most common infections in patients with HLH. Two patients had solid tumors (breast cancer and adenocarcinoma of thyroid). Similar results were seen in patients of the control group, with 1 hematologic malignancy in 8 cases with carcinoma. Based on repeated positive results of fecal occult blood test, GI hemorrhage was seen in 7 patients. Hemophagocytosis was observed in the bone marrow smear of 9 patients (Supplementary Figure 1, available with the online version of this article). AE-ILD was found in 13 patients (Figure 1). Because of the limited number of HLH cases, only Fisher's exact test and the 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 higher for patients in the mortality group. HLH patients with the diagnosis of CADM (p = 0.039) were found to have more favorable short-term outcomes. 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 our knowledge, this is the first study to investigate the risk factors for HLH in patients with IIM, and potential factors affecting the short-term outcomes of these patients. Regarding HLH in autoimmune diseases, previous studies revealed that HLH was more commonly seen in systemic juvenile idiopathic arthritis, systemic lupus erythematosus (SLE), adult-onset Still disease (AOSD), and Kawasaki disease[20,21,22,23]. In our 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 of patients with HLH was significantly higher than that of 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 IIM, leaves the patients vulnerable to malignancy and infection. A 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 our study, which might have 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. 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 and recruitment and proliferation of macrophages through a vicious loop[24,25]. Overexpression of ferritin could also be induced by

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

Characteristics	HLH, n = 18	Non-HLH, n = 72	p	p-adjusted
Age, yrs, mean ± SD	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, mos, median (IQR)	3.0 (1.0–8.5)	3.0 (1.0–6.0)	0.857	1.000
Duration of diagnosis delay, mos, median (IQR)	2.0 (1.0–3.5)	2.0 (1.0–4.8)	0.815	1.000
On-admission disease activity, median (IQR)	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 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
RP	0 (0.0)	7 (9.7)	0.376	0.893
Muscle pain	9 (50)	28 (38.9)	0.391	0.893
Muscle weakness	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
GI 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, median (IQR)	185.5 (56.5–1401.8)	752.0 (109.8–3498.8)	0.101	0.423
LDH, u/l, median (IQR)	584.5 (356.0–733.8)	500.0 (345.0–782.8)	0.709	1.000
ESR, mm/h, median (IQR)	30.5 (8.0–40.3)	20.0 (10.3–34.0)	0.548	1.000
CRP, mg/l, median (IQR)	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, median (IQR)	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-Jo1	1 (5.6)	6 (8.3)	1.000	1.000
ACA	0 (0.0)	2 (2.8)	1.000	1.000
Comorbidities/harmful habits				
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
IG	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+DMARD	5 (27.8)	37 (51.4)	0.073	0.410
Steroid+IG	5 (27.8)	7 (9.7)	0.104	0.423

Table 2. Continued.

Characteristics	HLH, n = 18	Non-HLH, n = 72	p	p-adjusted
Steroid+DMARD+IG	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
IV 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

Data are n (%) unless otherwise indicated. HLH: hemophagocytic lymphohistiocytosis; IQR: interquartile range; RP: Raynaud phenomenon; P-adjusted: adjusted p value after false discovery rate correction; ILD: interstitial lung disease; AE-ILD: acute exacerbation of ILD; GI: gastrointestinal; CK: creatine kinase; LDH: lactate dehydrogenase; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; on-admission hyperferritinemia: on-admission serum ferritin ≥ 500 ng/ml; ANA: antinuclear antibody; ACA: anticentromere antibody; MMF: mycophenolate mofetil; DMARD: disease-modifying antirheumatic drugs; IG: immunoglobulin; DST-based antibiotics: antibiotics based on drug-resistant testing; IV: intravenous; prophylactic SMZ: prophylactic application of sulfamethoxazole; IIM: idiopathic inflammatory myopathy; DM: dermatomyositis; PM: polymyositis; CADM: clinically amyopathic dermatomyositis; NA: not applicable.

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

Risk Factors	p	OR	95% CI
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

HLH: hemophagocytic lymphohistiocytosis; DM: dermatomyositis; PM: polymyositis; CADM: clinically amyopathic dermatomyositis; AE-ILD: acute exacerbation of interstitial lung disease.

Table 4. Overview of infection subtypes within the case group and the controls.

Infection Subtypes	Case Group, n = 18	Controls, n = 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%)
Viral infection	1 (5.6%)	1 (1.4%)

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 the development of secondary HLH requires more attention and further research.

Infection occurred in 15 (83.3%) of the 18 patients with HLH in our study, and was proved to be a risk factor for HLH in patients with DM, PM, or CADM. Multiple infectious agents including virus, bacteria, fungus, and parasite have been known to induce secondary HLH in patients with various diseases. Infection accounted for about 30% of all-cause secondary HLH in multiple studies[26,27,28]. However, in a Spanish study centered on patients receiving potent immunosuppressive treatment, two-thirds of patients with secondary HLH had suspected infectious triggers[29]. It was theorized that immunosuppressive medications contributed to a higher incidence of infection in that study. Multiple hypotheses exist for mechanisms of infection-related HLH. Suspected mechanisms include uncontrolled macrophage and T cell activation, acquired defects in cytotoxicity, cytokine storm, and TLR-related innate immune activation. Massive cytokine release was commonly seen in infection[30], and was hypothesized to impair natural killer (NK) cell cytotoxicity. In addition, several infectious agents were capable of suppressing cytotoxic T lymphocyte 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 proinflammatory cytokines[31]. A few TLR had also been identified in humans or mice as responding to microbial antagonists and subsequently driving innate immune response[32].

One study found that elevated systemic evaluating score was correlated with development of secondary HLH, or macrophage activation syndrome, in patients with AOSD[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. 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 analog scales involving different organs or systems[35]. 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 patients with IIM. Creatine kinase was not an appropriate evaluating tool for similar consideration. Under this circumstance, the integrity of the MYOACT score would alleviate the heterogeneity of different subtypes and reflect 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

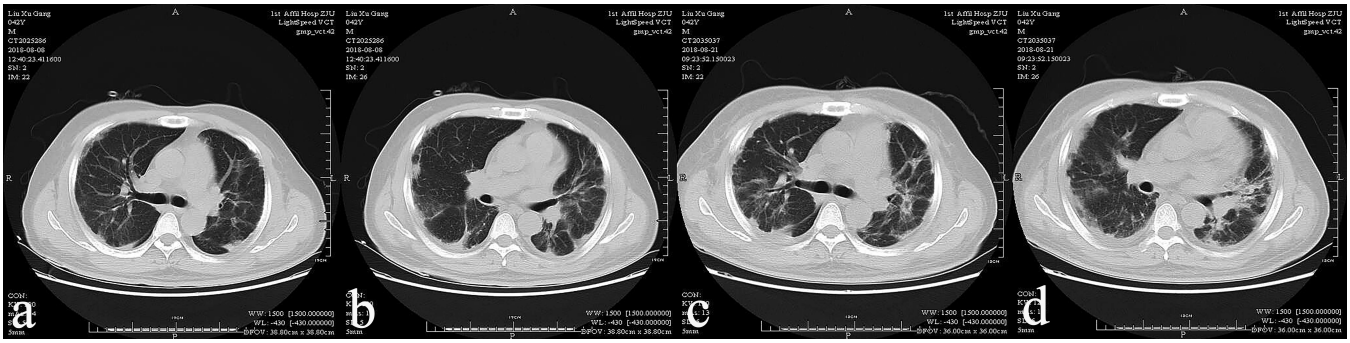

Figure 1. AE-ILD of a patient with HLH in this study (a/b: before exacerbation; c/d: after exacerbation). AE-ILD: acute exacerbation of interstitial lung disease; HLH: hemophagocytic lymphohistiocytosis.

history studies[34]. And some assessments such as manual muscle testing could not fully evaluate muscle impairment by assessing only the strength, not the endurance of the muscle[35]. The MYOACT score was also found to be consistent with the inflammatory level of patients with IIM and would thus reflect the autoimmune abnormality in IIM[36,37,38]. The correlation between disease activity and secondary HLH also indicated the potential role of autoimmune abnormality in the development of HLH in patients with IIM.

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. This is the first study, to our knowledge, to identify exacerbation of ILD as a risk factor for secondary HLH. The relevance between AE-ILD and development of HLH might reside in their overlapped pathological mechanisms. Macrophage activation and massive release of cytokines were suspected of promoting secondary HLH. Several studies had observed significant elevation of cytokines including interleukin (IL)-6 and IL-8, and M2 macrophage activation in patients with ILD exacerbation, and this elevation was found to be associated with worse outcomes[39,40,41,42,43]. 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 CTD-related ILD might, to some extent, be viewed as a reflection of aggravation of autoimmune abnormality. In that case, AE-ILD indicated an underlying deterioration of autoimmune abnormality in the development of HLH in the setting of CTD.

Comorbid illnesses including infection were found to play a contributing role in unfavorable outcomes of patients with secondary HLH[28,44]. Although patients with bacterial infection all experienced unfavorable outcomes, no significant correlation was identified between infectious agents and short-term outcome, possibly owing to the small sample size in our study. Secondary HLH patients with CADM seemed to have more favorable outcomes 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, a higher level of on-admission disease activity, which was evaluated by MYOACT score and reflected the severity of IIM, was also found in HLH patients with unfavorable short-term outcomes. Despite the negative result after FDR correction, the roles of on-admission disease activity, bacterial infection, and diagnosis of CADM deserve further verification in a larger cohort.

The high mortality rate of patients with HLH (77.8%) also precipitated review of therapeutic strategy. Frequent infections led to use of antibiotics and antifungal therapy, and prevented us from using stronger chemotherapy or immunotherapy in clinical practice. MMF treatment was not found to improve the outcomes of patients with HLH partly due to its rare application in patients with secondary HLH, 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, 2 SLE patients with secondary HLH were reported to be successfully treated with MMF, indicating the potential of MMF to cure secondary HLH in patients with CTD[46,47]. The significance, however, vanished after adjusting for factors including infection and disease activity. The unclarified but possible role of MMF in preventing and treating secondary HLH hence requires further study in a larger cohort. 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 antibacterial treatment in prevention of secondary HLH. Despite the active treatment targeting infection, the outcome of HLH patients was far from promising. Treatment of secondary HLH depends on the suppression of hyperinflammation and targeting of the underlying disease. Application of stronger chemotherapy or immunotherapy, based on the HLH-94 and HLH-2004 protocols[14,48], was deemed essential in spite of the frequent complication of infection. However, Kumar, *et al* questioned the effect of HLH-2004 in secondary HLH and proposed a modified therapeutic approach including anakinra, intravenous immunoglobulin, steroids, cyclosporine, and tocilizumab[49]. Continuous studies of the pathogenesis of secondary HLH also suggested potential effects of therapies targeting interferon-γ, CD52, and Janus kinase pathways, among others[50].

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

Characteristics	Mortality Group, n = 14	Survival Group, n = 4	p	p-adjusted
Age, yrs, median (IQR)	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, mos, median (IQR)	4.0 (2.0–16.8)	1.0 (1.0–4.0)	0.101	0.612
Duration of diagnosis delay, mos, median (IQR)	2.5 (2.0–3.8)	1.0 (1.0–4.0)	0.192	0.864
On-admission disease activity, median (IQR)	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 ≥ 500 ng/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 < 90 g/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 ≤ 1.5 g/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
GI 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
Viral 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
IG	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+DMARD	3 (21.4)	2 (50.0)	0.533	1.000
Steroid+IG	4 (28.6)	1 (25.0)	1.000	1.000
Steroid+DMARD+IG	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
IV 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

Data are n (%) unless otherwise indicated. P-adjusted: adjusted p value after false discovery rate correction; IQR: interquartile range; ILD: interstitial lung disease; NA: not applicable; AE-ILD: acute exacerbation of interstitial lung disease; GI: gastrointestinal; MMF: mycophenolate mofetil; IG: immunoglobulin; DMARD: disease-modifying antirheumatic drugs; DST-based antibiotics: antibiotics based on drug-resistant testing; IV: intravenous; prophylactic SMZ: prophylactic application of sulfamethoxazole; IIM: idiopathic inflammatory myopathy; DM: dermatomyositis; PM: polymyositis; CADM: clinically amyopathic dermatomyositis.

The most significant limitations of our study were the retrospective and observational design and the small sample size. Two variables in the diagnostic criteria of HLH (i.e., soluble CD25 and NK cell activity) were not measured because of their unavailability. The incidence of secondary HLH in our study might therefore be underestimated. Several myositis antibodies (i.e., antimelanoma differentiation–associated gene 5 antibody) were not included in the study owing to their absence in over

two-thirds of the patients involved and the retrospective design. Finally, we failed to provide valid suggestions for treatment of secondary HLH in patients with IIM owing to the serious heterogeneity of therapeutic regimens and the rarity of secondary HLH in these patients.

In patients with IIM, 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 the development of HLH in the setting of CTD. The role of higher on-admission disease activity, bacterial infection, and diagnosis of CADM in the short-term outcomes of patients with secondary HLH requires further study.

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ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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