

EDITORIAL

Haemophagocytic lymphohistiocytosis: from diagnostic challenges to predictive possibilities

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In 1832 Thomas Hodgkin described ‘*some morbid appearances of the absorbent glands and spleen*’ in seven individuals with enlargements of lymph nodes and spleen without inflammation or other significant pathological findings.¹ His initial description resolved into a multiplicity of pathological entities over the years.

Indeed, among this wide group of disorders, the *leucose, syphilitic and tuberculous lymphadenitis and lymphosarcoma* were isolated by Kundrat only in 1893. Sternberg then established the histological features of *lymphadenoma verum* in 1898 (followed by Reed in 1902), but other similar entities remained without a clear definition for years, and all together they were put into the group of the ‘atypical Hodgkin’s diseases’.

Only 40 years later the possibility that some of those cases represented a select group of histiocytic disorders was raised by Scott and Robb-Smith.² Following their first clinical observations on three cases which dated back to 1936, the authors tried to give a comprehensive definition of a subgroup of such disorders.

In the *Lancet* article issued in 1939, they reported four additional cases of what they histologically defined as *histiocytic medullary reticulosis*. This histological entity was associated with a typical clinical course: ‘[. . .] fever, wasting and generalised lymphadenopathy are associated with splenic and hepatic enlargement and in the final stage jaundice, purpura and anemia with profound leukopenia may occur. Post-mortem examination shows a systematised hyperplasia of histiocytes actively engaged in phagocytosis of the erythrocytes’.²

They referred to a group of diseases ‘[. . .] characterized by a progressive cellular hyperplasia throughout the hemopoietic and lymphatic tissue; these conditions differ from one other by virtue of the histological type or types of proliferated cells but owe their interrelation to the common mesenchymal origin of these cells. In some instances the proliferation has the characteristics of neoplasia, in others it does not transcend hyperplasia; but in all the change occurs in a systematised manner throughout the lymphoreticular tissue’.

Other similar descriptions followed later on, by Anderson (1944) and Asher (1946), until Farquhar and Claireaux³ in 1952 described a fatal disorder of the reticulo-endothelial system characterized by proliferation of histiocytes in solid organs and phagocytosis of blood cells occurring in infant siblings which they termed *familial hemophagocytic reticulosis*. On the basis of their studies and the previous evidence, the authors tried to gather the clinical and laboratory findings which they observed both in adults and in children into a definition of a single syndromic entity: ‘[. . .] a rare and invariably fatal condition, characterized by progressive erythropenia with or without depression of the circulating granulocytes and platelets despite a highly reactive marrow [. . .]. The liver, spleen and lymph nodes are enlarged, general intoxication is profound, and there may be a relapsing fever. The course seldom exceeds a few months, and terminally jaundice and purpura may appear’.³

Over the years, the multifaceted spectrum of this histological and clinical entity has had different definitions including the term ‘macrophage activation syndrome,’ used when the disease occurs in patients affected with rheumatological disorders, such as juvenile idiopathic arthritis.⁴ The Histiocyte Society proposed finally the term haemophagocytic lymphohistiocytosis (HLH) in 1991, and the related classifications and diagnostic guidelines, subsequently updated in 2004.⁵

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Similarly to other inflammatory and autoimmune conditions, a genetic predisposition has been supposed also for HLH: it has been observed that the predominant cause of some secondary forms of adult HLH differs in different countries, thus suggesting a specific genetic background or different triggering infectious agents according to the geographical localization.⁴

Most important, a clear distinction between primary and secondary forms of HLH has been established following the discovery of perforin gene mutations in 1999 by Stepp et al.⁶ Other gene products with a critical role for the proper functions of the cytotoxic natural killer (NK) granules were then identified and added further insights into the pathogenesis of primary HLH forms, whose onset is observed in the paediatric age group.

Secondary HLH forms may manifest as potential complications during the clinical course of infections and malignancies, or may represent the initial manifestation (or a life-threatening complication) of some autoimmune, rheumatic and autoinflammatory disorders.

More than 30 systemic or organ-specific autoimmune rheumatic diseases have been related to HLH so far, but a closer relationship and a more frequent association has been described in the cases of systemic lupus erythematosus (SLE) and adult-onset Still disease.⁴

Both primary and secondary forms share a defect in granule-mediated cytotoxicity, which usually contributes to maintain the dendritic cell homeostasis and to restrict T cell activation by antigen presentation. Antigen presentation is extremely enhanced in HLH and has been postulated as one of the causal mechanisms responsible for the macrophage activation.⁴

The presence of an uncontrolled activation of histiocytes and macrophages, engulfed with other haematopoietic elements detectable in the bone marrow, lymph nodes, spleen and other organs, is indeed a peculiar hallmark of HLH, together with a stormy, exaggerated immunological response that is responsible for life-threatening organ damage and high mortality rate, analogously to what is observed in severe sepsis and septic shock.⁷

As a matter of fact, the onset with fever, hypotension and progressive multi-organ failure, the presence of hypercytokinemia (interleukin (IL)-1, IL-4, IL-6, IL-8, IL-10, tumour necrosis factor alpha (TNF α), interferon gamma (INF γ), the activation of lymphocytes and macrophages, and a concomitant decreased NK-cell activity widely justify the clinical and laboratory overlap between HLH and severe sepsis.

Similarly, the detection of extremely high levels of ferritin is common to both conditions and has been recently proposed as a pivotal common denominator in severe sepsis/septic shock, secondary HLH (namely macrophage activation syndrome, associated with autoimmune rheumatic conditions), as well as in adult-onset Still disease and catastrophic antiphospholipid syndrome. The recent definition of the 'hyperferritinemic syndrome', including the above-mentioned four conditions, alludes to the supposed pathogenic role of high circulating levels of an aberrant ferritin isoform, which might contribute in activating macrophages and perpetuating the stormy immunological response.⁸

Starting from the assumption that hyperferritinemia-associated secondary forms of HLH can be successfully treated with a less immunosuppressive and cytotoxic approach than what is usually adopted in the primary forms, Demirkol et al.⁹ recently performed an observational cohort study to evaluate outcome in children with hyperferritinemia and secondary HLH overlapping with sepsis and multiple organ failure. They compared children who received plasma exchange (PE) with intravenous immunoglobulin (IVIG) or methylprednisone or both, with those who received PE with the HLH-94 protocol. The use of PE and methylprednisone or IVIG was associated with an improved survival compared to PE and dexamethasone and/or cyclosporine and/or etoposide. Similar recent evidence for a more effective and less cytotoxic approach supports the concept that in some cases of secondary HLH forms the immune system is only transiently altered and may recover once the initial stimulus is overcome, such as following an effective antibacterial therapy in the case of a reactive infection-related HLH.

Given the different aetiologies and the pleomorphic manifestations of HLH, to perform a correct diagnosis is often challenging but nonetheless crucial, because it significantly influences the therapeutic approach and the outcome of the disease. In this regard, the search for predictors of the response to treatment in the different forms of HLH is currently a matter of active debate and intense research.

In the same issue of this journal, Takahashi and colleagues¹⁰ reported seven cases of acute lupus haemophagocytic syndrome (ALHS) and performed a literature review and meta-analysis, aiming to identify factors useful to predict the response to treatment of ALHS, including corticosteroid monotherapy and cyclosporine A (CsA).

ALHS was first described in 1991 by Wong et al.,¹¹ who referred to an unusual presentation of SLE characterized by acute and severe pancytopenia associated with reactive haemophagocytosis, which they observed in a series of patients from Hong Kong hospitals. In the six patients described there was no evidence of an underlying infection and the response to steroids was dramatic.

Similarly, between 2005 and 2013 Takahashi et al.¹⁰ reported seven patients diagnosed with ALHS, among whom five experienced ALHS as the first manifestation of SLE. Interestingly, cases with low anti-double-stranded DNA (anti-dsDNA) antibody titres tended to have more severe hyperferritinemia, and a negative and significant correlation between anti-dsDNA antibodies and levels of serum ferritin was finally found.

The authors compared their results with 93 other cases of ALHS detected in the literature via a Medline research performed from 2001 through 2014. Cases complicated with infections or malignancies were excluded. Once again, a negative correlation between serum ferritin and anti-dsDNA antibodies was confirmed, also among the published reports. One of the possible interpretations of these results might allude to the existence of two subtypes of ALHS characterized by two different pathological substrates: one with high ferritin-low anti-dsDNA antibodies, and the other with low ferritin and high antibody titres. The authors speculate that in the former type the activation of histiocytes is mainly due to the cytokine storm reflected by hyperferritinemia; in the latter type antibodies or immunocomplexes might be mainly responsible for the activation of histiocytes according to the pathogenic mechanism of autoimmune-associated haemophagocytic syndrome proposed by Kumakura in 2004.¹²

The meta-analysis performed in order to investigate the predictors of response to corticosteroid monotherapy showed that serum ferritin was not a significant predictive factor. In addition, low serum C-reactive protein levels and high haemoglobin concentrations before the treatment resulted in significant and independent predictors of the response to corticosteroid monotherapy, alluding to a more probable effective response to this therapeutic schedule in the case of a milder phenotype of the disease.

On the contrary, the analysis of prediction of response to CsA showed that among the variables at diagnosis immediately before treatment of HLH, serum levels of ferritin were significantly higher in the CsA-responders than non-responders cases. High ferritin levels and low leucocyte count were

significant predictors of the response to CsA in ALHS. In these cases, the authors speculate that excessive cytokine production from pathogenic T cells could be the underlying pathological process, which resembles more the clinical and therapeutic behaviour of the severe familial forms of HLH and the macrophage activation syndromes related to juvenile idiopathic arthritis. To summarize, high ferritin levels and leucocytopenia could reflect a more severe phenotype and a possible response to CsA in ALHS, in case of a non-response to corticosteroids monotherapy.

Undoubtedly, further confirmations of these results in larger populations are needed, given the small sample size examined and the limited statistical analyses.

However, these data open an intriguing scenario in the management and prediction of outcome and relapse of such conditions, or at least in some of the HLH forms. Indeed, this evidence is gathered together with the recent observations on the haematological or liver signs such as steady or increased concentrations of ferritin, soluble CD25 and soluble CD163, as markers of possible disease relapse.^{13,14}

In conclusion, given the increasing insights into the pathogenesis of several autoimmune and autoinflammatory disorders, and thanks to the rapid and continuous improvement of the diagnostic techniques, we are currently living in the era of the *prediction* of autoimmune diseases.¹⁵⁻¹⁹ Whereas the autoimmune (or autoinflammatory) disease has already been manifested, the current and future challenge is to detect predictive markers of the response to treatment and of relapse, the latter becoming more and more sophisticated and personalized, according to the new pathogenic acquisitions.

We have briefly revisited the history of HLH since the first pathological observations in order to underline as the progression of the pathogenic insights proceeds accordingly to the scientific technical advances, especially in the case of rare conditions. HLH is a valid example of the plenty of still-open challenges we are facing in the field of autoimmune and autoinflammatory diseases. The Takahashi et al.¹⁰ study results allude to possible different sub-phenotypes of the same disease, which differently respond to a different therapeutic schedule. The identification of predictive markers for the treatment response and disease outcome helped in formulating this hypothesis, which still requires further confirmation.

In their first description of *histiocytic medullary reticulosis*, Scott and Robb-Smith stated that *'progress will only be made when sufficient 'atypical'*

cases have been collected to be submitted to histological analysis and classification'.² In the case of HLH we currently are able to distinguish in most cases primary from secondary HLH^{20,21} forms, we have diagnostic techniques useful to try to detect the trigger agents in the secondary forms, we have a set of diagnostic criteria²² which include peculiar disease markers, but still the sensitivity and specificity of these criteria remain untested, the rate of misdiagnosed and undiagnosed cases is unknown, and the predictors of response to treatment and relapse need to be verified. In this view, HLH is a fertile field for basic and clinical research, which requires further efforts from different and integrated multidisciplinary approaches, in order to give to what is nowadays unclear to our eyes and then defined 'atypical', its proper correct denotation in the future.

The dream of prediction entails among others a microarray technology, as it has been already described in SLE.²³ Employing a drop of blood and bioinformatics will enable us to determine disease genetic markers, as well as cytokines expression profiles, combined with data on environmental factors exposure (i.e. Epstein-Barr virus (EBV)) to calculate the risk of disease development and responding rates to complex therapies.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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