Secondary haemophagocytic lymphohistiocytosis in COVID-19: correlation of the autopsy findings of bone marrow haemophagocytosis with HScore

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ABSTRACT

Background Secondary haemophagocytic lymphohistiocytosis (sHLH) is characterised by a hyper activation of immune system that leads to multiorgan failure. It is suggested that excessive immune response in patients with COVID-19 could mimic this syndrome. Some COVID-19 autopsy studies have revealed the presence of haemophagocytosis images in bone marrow, raising the possibility, along with HScore parameters, of sHLH.

Aim Our objective is to ascertain the existence of sHLH in some patients with severe COVID-19.

Methods We report the autopsy histological findings of 16 patients with COVID-19, focusing on the presence of haemophagocytosis in bone marrow, obtained from rib squeeze and integrating these findings with HScore parameters. CD68 immunohistochemical stains were used to highlight histiocytes and haemophagocytic cells. Clinical evolution and laboratory parameters of patients were collected from electronic clinical records.

Results Eleven patients (68.7%) displayed moderate histiocytic hyperplasia with haemophagocytosis (HHH) in bone marrow, three patients (18.7%) displayed severe HHH and the remainder were mild. All HScore parameters were collected in 10 patients (62.5%). Among the patients in which all parameters were evaluable, eight patients (80%) had an HScore >169. sHLH was not clinically suspected in any case.

Conclusions Our results support the recommendation of some authors to use the HScore in patients with severe COVID-19 in order to identify those who could benefit from immunosuppressive therapies. The presence of haemophagocytosis in bone marrow tissue, despite not being a specific finding, has proved to be a very useful tool in our study to identify these patients.

INTRODUCTION

The outbreak of the novel coronavirus pandemic is supposing a medical challenge worldwide. The main clinical features of COVID-19 vary from asymptomatic or mild symptoms such as fever, cough and myalgia to severe pneumonia with acute respiratory distress syndrome (ARDS), requiring at times ventilatory support.[1–5] Severe patients also display signs of high systemic inflammatory response with altered pattern of inflammatory chemokines and cytokines and high ferritin levels, known as cytokine storm.[4 6]IL-1/IL-6 pathway dysregulation seems to

play a crucial role in the pathogenesis of the severe complications of patients with COVID-19, as has been suggested by the favourable evolution of some patients treated with their receptor antagonists.[7 8]

Secondary haemophagocytic lymphohistiocytosis (sHLH), cytokine release syndrome and macrophage activation syndrome are overlapping syndromes characterised by an activation of lymphocytes and macrophages with a subsequent excessive immune response, cytokine storm and haemophagocytosis, which leads to multiorgan damage.[9–12]The most frequent triggers of this syndrome are infections and malignancies. Among viral infections, the most frequent causative agent is Epstein-Barr virus, but other viruses like respiratory syncytial virus, rotavirus and adenovirus infections have been reported.[13–15]

Clinical presentation of sHLH consists of fever, hepatosplenomegaly, cytopenias, acute liver failure, high ferritin and CD25 soluble (sCD25), coagulopathy, dermatologic manifestations as Kawasaki-like syndrome and neurological symptoms.[10] Around 50% of patients develop respiratory symptoms.[15] Diagnosis of sHLH is based on clinical and laboratory criteria. Until recently, the criteria were extrapolated from the primary haemophagocytic lymphohistiocytosis (HLH2004 criteria).[16] However, in 2014, Fardet _et al_ proposed a new score validated in patients with reactive HLH, called the HScore.[17] It is based on nine variables: three clinical features, five laboratory parameters and one pathological finding, which is the presence of haemophagocytosis in bone marrow.

In accordance, some authors suggest that the immune response seen in COVID-19 could mimic sHLH and, therefore, recommend using the HScore to identify patients who could benefit from immunosuppressive therapies.[18]

The presence of haemophagocytosis in bone marrow could raise the possibility of sHLH in patients with severe COVID-19. Wood _et al_, based on intensive care unit (ICU) patients' study, have considered that HScore has limited application in severe patients with COVID-19. Nevertheless, they calculated HScore without bone marrow biopsy.[19]

Various autopsy series on patients with COVID-19 have begun to emerge in the literature. Only few of them include the study of bone marrow tissue for the presence of haemophagocytosis. Some of these studies, based on the hypothesis above described,

correlate this finding, along with HScore, with a possible hyper-inflammatory status and hypercytokinemia/sHLH, reporting contradictory results about the presence of haemophagocytosis and the convenience of using the HScore in these patients due to a potential lack of sensitivity.[20–25] In order to ascertain the existence of sHLH in patients with COVID-19, we report the histological findings from 16 COVID-19 autopsies, one of the largest series published until now that focuses on the presence of haemophagocytosis in bone marrow integrating these findings with clinical and laboratory criteria for sHLH diagnosis.

METHODS
Patient selection
We performed a single-centre retrospective analysis in 16 patients with COVID-19 disease in a tertiary care hospital. We considered as confirmed cases the patients with compatible clinical symptoms or a suggestive chest image and a positive reverse transcription-PCR for SARS-Cov-2 nucleic acid on upper respiratory swap.[26]

Clinical and laboratory data
We calculated the HScore for all patients. The HScore is freely available online (http://saintantoine.aphp.fr/score/). As previous recommendations for this score, we used a cut-off point of 169 points, corresponding to a sensitivity of 93% and a specificity of 86% for the diagnosis of sHLH.[17] Clinical evolution and laboratory parameters were collected from electronic clinical records. For the laboratory parameters, the value corresponding to the maximum score during evolution was considered. Organomegaly was obtained from physical exploration or from radiological findings. Missing data were scored as 0 points. The presence of haemophagocytosis was assessed postmortem in bone marrow tissue from autopsies, as explained below.

We did not calculate the HLH 2004 score because of the sCD25 and the natural killer (NK)-cell activity were not available in any patients.

Autopsy procedure
All autopsies were performed between 19 April and 4 June, when clinicians requested them with the consent of relatives, in patients with severe COVID-19 with unexpected unfavourable clinical course. They were conducted in a negative pressure room using personal protective equipment and performed according to a security protocol as previously reported.[27] We collected tissue from each organ according to our autopsy protocol,[27] which includes haematopoietic tissue. Collected tissues were fixed for 24 hours in 10% buffered formalin. After fixation, rib tissue was decalcified with formic acid for 24 hours before embedding in paraffin blocks. Multiple unstained 3–5 µm-thick sections were cut for H&E stain and immunohistochemistry. H&E staining was applied according to the standard protocols.

Immunohistochemistry
To identify the presence and the architectural distribution of the different haematopoietic series and lymphoid populations, immunohistochemistry was performed using a routine panel of antibodies against CD20 (clone L26, Agilent), CD3 (polyclonal, Agilent), CD4 (clone 4B12, Agilent), CD8 (clone C8/144B, Agilent), CD68 (clone PG-M1, Agilent), glycophorin C (clone ret40f, Agilent), CD61 (clone Y2/51, Agilent) and myeloperoxidase (polyclonal, Agilent). All of them were performed on an OMNIS (Agilent) automated stainer, except for CD71 (MRQ-48, Roche), which was performed on the Benchmark Ultra Ventana

systems (Roche). CD68 immunohistochemical stains were used to highlight the histiocytes and haemophagocytic cells. Double staining glycophorin C/CD68 was performed in order to identify more precisely haemophagocytic cells.

Bone marrow histiocytic quantification
Only histiocytes showing engulfment of one or more nucleated host blood cells, including plasma cells and lymphocytes, were counted as haemophagocytosis. Moreover, morphological evaluation of histiocytic hyperplasia with haemophagocytosis (HHH) was graded in bone marrow tissue based on Suster et al score[28] such as: mild HHH (haemophagocytosis was present only when searched for in several high-power fields (HPF)), moderate (haemophagocytosis was present in one to three cells per HPF) and severe (haemophagocytosis was present more than three cells per HPF). This classification considers as pathological HHH only those cases demonstrating moderate to severe haemophagocytic activity. Nevertheless, since the HScore requires only the presence of haemophagocytosis in bone marrow to meet this parameter,[17] we used this classification in order to establish a possible association between sHLH diagnosis and HHH degree. We also calculated the percentage of histiocytes in each sample by counting the number of histiocytes stained with CD68 in 500 bone marrow cells. In normal conditions, the histiocytic cellularity in bone marrow is inconspicuous. Histiocytic hyperplasia was considered in our study when the percentage was 5% or superior.

Moreover, following the Gars et al study,[29] we searched for the presence of multiple nucleated cells within a single haemophagocyte in each case.

RESULTS
Clinical findings
Sixteen patients were collected. All of them had positive SARS-CoV-2 PCR. The median age of our cohort was 64·5 years (range 57–73). The 75% of patients were men. The symptoms at diagnosis were fever (87·5%), cough (56·3%), dyspnoea (37·5%), gastrointestinal symptoms (31·3%) and asthenia (18.8%). Other less common symptoms were chest pain (12·5%), anosmia (6) and odynophagia (6.3%). All patients suffered from ARDS and 14 (87·5%) required orotracheal intubation and admission to an ICU. Nine patients (56·3%) suffered acute renal injury and 18.8% underwent dialysis treatment.

The median time from COVID-19 diagnosis to death was 33 days (range 24–42). From the 16 patients, 11 (68·8%) negativised SARS-Cov-2 PCR in a median 23 days (range 20–36). In three patients, control sequential SARS-Cov-2 PCR was not performed.

Following the clinical protocol, lopinavir/ritonavir was prescribed in 11 patients (68·8%), hydroxychloroquine in 14 patients (87·5%) and azithromycin in 12 patients (75%). Corticosteroids were administered in 14 patients (87·5%) and tocilizumab in 13 patients (81·3%). We reviewed blood, respiratory and urinary cultures. In nine patients (56·3%), other infectious pathogens were isolated. These patients received concomitant antimicrobial treatment.

We reviewed the prior relevant comorbidities to COVID-19 diagnosis, which were arterial hypertension in six patients (37·5%), hypercholesterolemia in five patients (31·2%), cardiovascular disease in five patients (31·2%), sleep apnoea syndrome in two patients (12·5%), cognitive impairment in two patients (12·5%) and hepatitis C virus hepatopathy in one patient (6·3%). Three patients had oncohematological history and only
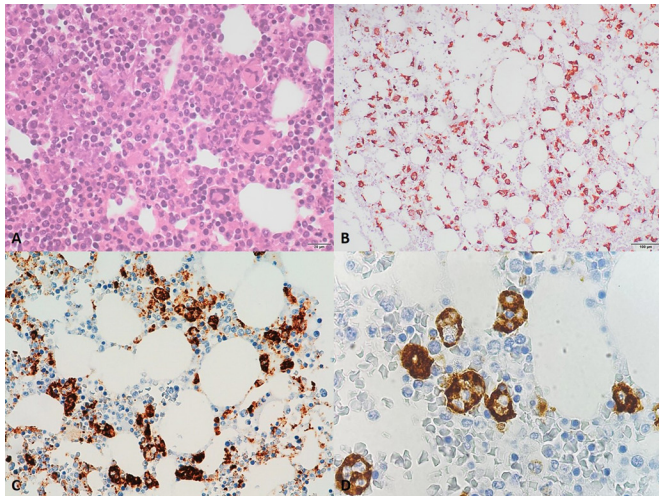
Figure 1 Bone marrow pathological findings. (A) (H&E stain, 40×) High- power view of haemapoietic cells in bone marrow. (B) (CD68 stain. 10×) Low-power view of bone marrow with histiocytic hyperplasia with haemophogocytosis. (C) (CD68 stain, 40×) High power view of severe HHH in born marrow. (D) (CD68 stain, 100x) high-power view of haemophagocytosis images. HHH, hyperplasia with haemophagocytosis.

one of them was in treatment with intensive chemotherapy due to myeloproliferative neoplasm in blast phase.

Autopsy findings

Pathological autopsy findings are described in table 1.

Regarding to bone marrow tissue, HHH was observed in all cases in varying degrees (Figure 1).

Histiocytic hyperplasia was easily observed in all cases but one, with percentages, ranged from 7% to 13%.

Eleven patients (68.7%) displayed moderate HHH, 3 patients (18·7%) displayed severe HHH and 2 (12·5%) displayed mild HHH. The maximum number of haemophagocytosis was objectified in case 12 (4·7 haemophagocytosis/HPF). These specific values are reflected in table 2, along with the remaining parameters of HScore.

Immunohistochemical study also demonstrated CD8+ predominance lymphocytosis as a common finding in all cases. The histological and immunohistochemical features in bone marrow tissue were very similar in all the cases. The overall cellularity was increased with left shift deviation from granulocyte series and hyperplasia of megakaryocytic series with reactive changes was also observed.

The presence of more than one nucleated cell within a single haemophagocyte was observed in all but two cases (cases 13 and 16). These two cases were the ones that displayed mild HHH.

Lung tissue from each lobe was collected. In all cases, lungs had a consolidated appearance, especially the lower lobes. Histologically, the main finding in lung parenchyma was diffuse alveolar damage (DAD), both in its proliferative phase (10/16 patients) and in its exudative phase (6/16 patients). Four patients also showed DAD in advanced fibrous phase. One patient showed interstitial lymphoid pneumonitis. In all cases, primary cause of death was attributed to the severe lung damage.

Spleen tissue could be evaluated only in 13 patients, due to autolytic changes in three of them. The main finding in spleen tissue was depletion of white pulp, seen in 11/13 patients. Histiocytic hyperplasia was observed in 10 patients. Haemophagocytosis of nucleated cells was seen in four patients. CD8+ predominance lymphocytosis was also observed in splenic tissue, most of them in a lower degree compared with bone marrow tissue. Only one case (case 9) showed severe CD8+ lymphocytosis. Regarding other findings, extramedullary haematopoiesis was evidenced in five patients.

Table 1 Pathological findings in lung, spleen and lymph node

Case	Lung tissue findings	Spleen tissue findings	Lymph node tissue findings
1	DAD (fibrous phase)	Necrotic tissue	Not collected
2	DAD (exudative phase)	Necrotic tissue	Not collected
3	DAD (exudative phase)	Focal necrosis. Histiocytic hyperplasia	Not collected
4	DAD (proliferative phase)	Depletion of the white pulp. Haematopoiesis. Histiocytic hyperplasia. Haemophagocytosis.	Not collected
5	DAD (proliferative phase)	Histiocytic hyperplasia. Erytrophagocytosis.	Plasmocytosis. Erythrophagocytosis.
6	DAD (proliferative phase)	Widely necrotic. Depletion of the white pulp.	Lymphoid depletion. Erythrophagocytosis.
7	DAD (proliferative phase)	Depletion of the white pulp. Histiocytic hyperplasia. Severe haemophagocytosis.	Not collected
8	DAD (exudative phase)+acute bronchopneumonia	Depletion of the white pulp. Haematopoiesis. Histiocytic hyperplasia.	Lymphoid depletion. Plasmocytosis. Erythrophagocytosis.
9	DAD (proliferative phase)	Haemorrhage. Depletion of the white pulp. Histiocytic hyperplasia. Erythrophagocytosis.	Lymphoid depletion. Plasmocytosis. Erythrophagocytosis.
10	DAD (proliferative phase)	Focal necrosis. Haemorrhage. Depletion of the white pulp.	Not collected
11	DAD (proliferative phase)	Focal necrosis. Haemorrhage. Depletion of the white pulp. Haematopoiesis.	Not collected
12	Interstitial lymphoid pneumonitis	Depletion of the white pulp. Histiocytic hyperplasia. Haemophagocytosis.	Not collected
13	DAD (exudative phase)	Depletion of the white pulp. Histiocytic hyperplasia. Haemophagocytosis.	Lymphoid depletion. Erythrophagocytosis.
14	DAD (exudative, proliferative and fibrous phase).	Depletion of the white pulp. Haematopoiesis. Histiocytic hyperplasia.	Not collected
15	DAD (exudative, proliferative and fibrous phase)	Depletion of the white pulp. Haematopoiesis. Histiocytic hyperplasia.	Lymphoid depletion
16	DAD (proliferative and fibrous phase)	Necrotic tissue	Not collected

DAD, diffuse alveolar damage.

Table 2 HScore parameters and bone marrow pathological findings

| Patient	Basal IS*	Hepatomegaly	Splenomegaly	Maximum body T (°C)	Lowest Hb*	Lowest WBC†	Lowest platelets†	Maximum ferritin‡	Maximum triglycerid§	Lowest fibrinogen¶	Max GOT**	Haemophagocytosis in BM	N° histiocytes††	Haemophagocyt images per HPF	HScore points‡‡	% Probability HScore
1	No	No	No	<38.4°C	8.8	6.4	48.4	2700	4.8	170	215	Yes	60 (12%)	1.3	207/337	92
2	No	No	No	<38.4°C	10.4	8.48	177	NA	NA	530	48	Yes	50 (10%)	2.3	54/223	0.09
3	No	No	No	>38.4°C<39.4°C	7.3	2.96	71.9	1988	6.45	180	101	Yes	36 (7%)	1.9	196/337	85
4	No	No	No	>38.4°C<39.4°C	7.9	2.4	17.6	NA	NA	140	116	Yes	50 (10%)	1.4	151/223	26.3
5	No	No	No	<38.4°C	7	2.35	41	1436	4.16	160	74	Yes	67 (13%)	3.2	182/337	70.97
6	No	No	No	>38.4°C<39.4°C	8.1	4.69	53	2163	NA	100	82	Yes	55 (11%)	3.2	186/273	75.8
7	No	No	No	>38.4°C<39.4°C	8.7	2.8	106	795	NA	120	54	Yes	55 (11%)	1.4	151/273	26.3
8	Yes	No	No	>38.4°C<39.4°C	10.1	2.8	170	NA	NA	360	22	Yes	50 (10%)	1.3	86/223	0.63
9	No	No	No	>38.4°C<39.4°C	7.6	4.07	60.9	1689	5.1	160	67	Yes	62 (12%)	1.7	215/337	94.97
10	No	No	No	≥39.4°C	8.3	4.02	79.7	516	9.31	140	124	Yes	50 (10%)	2.2	212/337	94
11	No	No	No	>38.4°C<39.4°C	7.6	8.7	120	1454	1.2	360	58	Yes	59 (12%)	1.8	87/337	0.67
12	No	No	No	≥39.4°C	12.5	10.4	31.6	714	0.49	160	87	Yes	60 (12%)	4.7	133/337	10.4
13	Yes	Yes	Yes	≥39.4°C	6.5	0.01	8	2494	NA	290	85	Yes	45 (9%)	0.6	239/273	98.8
14	No	Yes	No	≥39.4°C	6.3	2.79	30.8	666	4.24	280	124	Yes	34 (7%)	1.1	224/337	97.06
15	No	Yes	No	<38.4°C	8.1	5	80.3	1759	3.28	110	57	Yes	43 (9%)	1.6	185/337	74.64
16	No	Yes	No	≥39.4°C	6	3.93	60	6900	5.12	100	129	Yes	40 (8%)	0.5	304/337	99.97

*g/dL.
†10³/μL.
‡ng/mL.
§mmol/L.
¶mg/dL.
**U/L.
††N° histiocytes per 500 nucleated cells.
‡‡Score points/ maximal points for this patient with his/her available parameters.
BM, bone marrow; GOT, glutamic oxaloacetic transaminase; Hb, haemoglobin; HPF, high power field; IS, immunosuppression; NA, not available; WBC, white cell count.
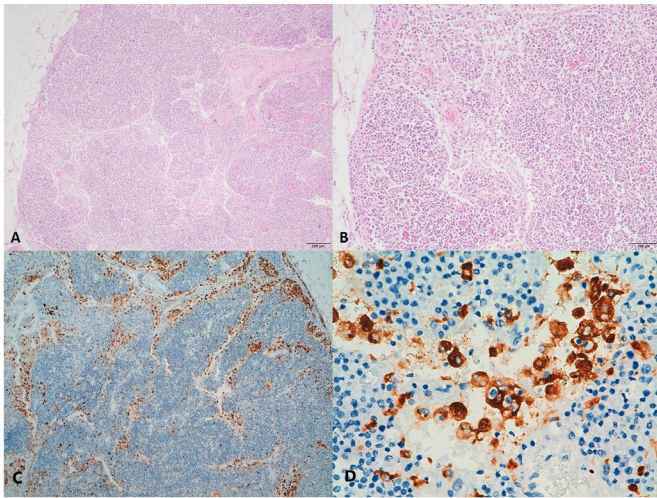
Figure 2 Lymph node tissue pathological findings. (A) (H&E stain, 4x) Low-power of view lymph node in which sinusal histiocytosis is remarkable. (B) (H&E stain, 10x) Low-power view of sinusal histiocytosis in lymph node. (C) (CD68 stain, 4x) Low-power of view of lymph node with sinusal histiocytosis. (D) (CD68 stain, 40x) High-power view of haemophagocytosis in lymph node.

Lymph node tissue was collected from six patients. All of them displayed small size. Lymphoid depletion with sinusal histiocytosis was a common finding (5/6 patients). Haemophagocytosis was observed in four patients (figure 2). Three patients showed an extended interfollicular plasmocytosis.

HScore

Parameters evaluated in each case and final HScore are described in table 2.

We could collect all HScore parameters in 10 patients (62.5%). In three patients, ferritin levels were missing (273/337 points were available), and in other three patients, triglycerides and ferritin levels were missing (223/337 points were available). Among the patients in which all parameters were evaluable, 8 patients (80%) had an HScore >169. Between the patients with a HScore <169, four were patients with missing data. sHLH was not clinically suspected in any case.

During COVID-19 evolution, a maximum body temperature >38·4°C was present in 12 patients (75%), splenomegaly in 1 patient (6·3%) and hepatomegaly in 4 patients (25%). Among laboratory parameters, 13 patients (81·3%) presented a haemoglobin level <9·2 g/dL, 10 patients (62·5%) a leucocyte count <5000×10⁹/L, 13 patients (81·3%) platelets <110×10⁹/L, 14 patients (87·5%) fibrinogen <250 mg/dL and all patients a maximum glutamic oxaloacetic transaminase (GOT) >30 U/L. Among the 10 patients with available triglyceride levels, hypertriglyceridemia >1·5 mmol/L was present in 8 patients (80%) and among the 13 patients with ferritin available, ferritin level >2000 ng/mL was present in 4 patients (30·8%).

All patients presented haemophagocytosis in bone marrow tissue. From the eight patients with all HScore parameters available and a total points 169, all but one displayed pathological (moderate to severe) HHH, six in moderate degree and one in severe degree. In all of them, more than one nucleated cell within a single haemophagocyte was observed. Of those eight patients, four of them would not reach a score of 169 without the item of haemophagocytosis in bone marrow.

DISCUSSION

The differential diagnosis between a severe infection and HLH can be challenging since an infection can precede and trigger HLH.[30] Furthermore, if there is not a high index of suspicion, the diagnosis of sHLH is often missed or delayed. Unless it is diagnosed early and an appropriate treatment is instituted, the mortality rate is high.[31]

It has been suggested that a subgroup of patients suffering from severe COVID-19 pneumonia could develop sHLH.[2 32 33] These patients meet the laboratory parameters necessary to the diagnosis of sHLH such as elevated CPR and hyperferritinemia and other clinical conditions including abnormal liver function and coagulopathy.[34] Previous related viruses as SARS-CoV and MERS-CoV showed similar clinical course than SARS-CoV-2, although in the first two, the proportion of patients with fatal evolution was higher, especially in MERS-COV.[35]

We calculated the HScore of 16 patients with COVID-19. In 10 of these patients (62·5%), all the parameters could be collected and HScore was evaluable. Among these, 80% had a global score >169 points. In those patients in which HScore was lower, in 66% not all parameters could be collected. Our results support the recommendation of some authors of suspecting hyperinflammation in patients with severe COVID-19 and using HScore in order to identify patients who could benefit from immunosuppressive therapies.[18] Haemophagocytic syndrome was not clinically suspected in any of our cases.

Leverenz et al preclude that HScore is not reliable in patients with COVID-19 due to its lack of specificity when assessing the leukocytopenia as it does not differentiate neutropenia from lymphopenia and the absence of extremely elevated ferritin levels in patients with COVID-19.[36] It is true that patients with COVID-19 have some differences with patients with sHLH, being the most important the absence of lymphadenopathy or splenomegaly, the absence of hypofibrinogenemia and the not so extremely elevated ferritin levels.[3 4 19] However, based on our findings and previous reports, we consider that they share *core* similarities (presence of haemophagocytosis, raised ferritin levels) which reflect a common immune response in COVID-19 and sHLH driven by the macrophages. Moreover, the only treatment for COVID-19 with impact on mortality to date (dexamethasone) is one of the main agents included in HLH protocols.[37 38] We consider that HScore is a useful tool to identify this sHLH-like syndrome in COVID-19 patients.

McGonagle et al,[34] based on an immunopathology model, postulated that sHLH in COVID-19 pneumonia could be restricted to the pulmonary compartment, being considered an acute ARDS damage rather than a systemic macrophage activation, characteristic of sHLH. In our study, we found that not only pulmonary compartment was involved, but also reticuloendothelial organs. Therefore, our results contradict this hypothesis.

Regarding the presence of haemophagocytosis in bone marrow tissue, it has been reported that most of the patients have haemophagocytosis at the time of diagnosis of HLH.[39] Previous studies have attempted to establish the number of haemophagocytosis in bone marrow that could define a diagnosis of HLH.[20 31 40–43] Although there is not a consensus, the probability may be higher if severe haemophagocytosis is observed.[20] Gars et al[29] evaluated the presence of multiple nucleated cells within a single haemophagocyte in bone marrow as a possible morphological feature that may differentiate patients with pathological haemophagocytosis. In our study, the presence of multiple nuclei within the same histiocyte seems to be more related to the grade of HHH rather than to sHLH. It is known

that the demonstration on bone marrow aspiration/biopsy is not mandatory for the diagnosis of sHLH.[44 45] Nevertheless, it can be a useful tool, if the other characteristic criteria are not available in time to aid in immediate treatment-related decisions.[31] In our study, this parameter has been decisive at the time of calculating HScore in many patients[46] besides this phenomenon is not specific of HLH, as it can be seen in other conditions such as postblood transfusion, haemolysis, myelodysplasia/bone marrow failure or sepsis.[44–49] Therefore, it is mandatory to exclude all these conditions when haemophagocytosis is observed and HLH is suspected.

Few studies of COVID-19 autopsy series[21 23 24] have demonstrated the presence of haemophagocytosis in bone marrow in some of the patients. Most of them are based on small series or samples obtained from bone marrow aspiration or trephine biopsy. We present one of the largest autopsy series focusing on the study of very representative samples of bone marrow, taken from rib squeeze. Moreover, quantification of the grade of HHH and its correlation with the clinical and analytical features distinctive of HLHs HScore have not been done until the present study. Although haemophagocytosis was present in all the studied cases, meeting the pathological parameter of the HScore, our results show that the patients with higher probability of sHLH displayed severe or moderate HHH.

Regarding the limitations of the study, it must be considered that the clinical and analytical parameters taken from our patients correspond to their highest values, not to the time when they worsen clinically, since they were studied postmortem. Based on this observation, we recommend, when sHLH is suspected, to collect the laboratory and clinical parameters on a standardised basis.

In conclusion, in virtue of the high percentage of cases (80% of the evaluable cases) that had a global HScore >169 points observed in our study, the possibility of HLH secondary to COVID-19 must be considered, especially in severe patients. Therefore, it would be advisable to calculate the HScore in these patients in order to identify patients who may benefit from more intensified immunosuppressive therapy.

Bone marrow haemophagocytosis is frequent and, therefore, HLH may be underestimated when H-scores are calculated without a bone marrow biopsy, so in those patients with clinical suspicion of sHLH in which HScore is close to the 169 cut-off, bone marrow biopsy with immunohistochemical staining for histiocytic markers may aid in establishing the sHLH diagnosis.

Take home messages

- ► We are presenting one of the largest autopsy series demonstrating haemophagocytosis in bone marrow, added to the contribution of clinical data to integrate a possible diagnosis of secondary haemophagocytic lymphohistiocytosis (sHLH) in patients with COVID-19.
- ► All 16 patients had any degree of histiocytichyperplasia with haemophagocytosis in the bone marrow sample. Among evaluable patients for HScore, 80% had >169 points.
- ► In severe patients with COVID-19, it would be advisable to calculate the HScore in order to identify who may benefit from more intensified immunosuppressive therapy.
- ► In cases with total points close to 169, a bone marrow sample could help to establish the sHLH diagnosis.

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