Bone marrow haemophagocytosis indicates severe infection with severe acute respiratory syndrome coronavirus 2

Julia Swoboda,1 Daniel Wittschieber,1 Juliane Sanft,1 Sandra Kleemann,1 Stefan Elschner,1 Hannah Ihle,1 Michael Hubig,1 Mathias W Pletz,2 Gita Mall1 & Nikolaus Gassler3

1Institute of Forensic Medicine, Jena University Hospital, Jena, 2Institute for Infectious Diseases and Infection Control, Jena University Hospital, Jena, and 3Section of Pathology, Institute of Forensic Medicine, Jena University Hospital, Jena, Germany

Date of submission 25 August 2020
Accepted for publication 14 October 2020
Published online Article Accepted 17 October 2020

Swoboda J, Wittschieber D, Sanft J, Kleemann S, Elschner S, Ihle H, Hubig M, Pletz M W, Mall G & Gassler N (2021) Histopathology 78, 727–737. https://doi.org/10.1111/his.14281

Bone marrow haemophagocytosis indicates severe infection with severe acute respiratory syndrome coronavirus 2

Aims: Haemophagocytosis in the bone marrow of patients who have succumbed to coronavirus disease 19 (COVID-19) has not been widely studied. The aims of the present study were to perform morphological analyses and morphometry of haemophagocytosis in the bone marrow of patients with severe COVID-19, and to correlate the findings with the clinical course of the disease.

Methods and results: In this single-centre study performed at the University Hospital Jena, bone marrow specimens of 15 deceased patients who had experienced a severe course of COVID-19 were sampled from the vertebral column during autopsy.Slides of the bone marrow were stained with routine stains or immunohistochemically, and further examined for haemophagocytosis by the use of light microscopy. To substantiate the morphological findings, additional slides were stained for CD163 and morphometry was performed. In all bone marrow samples, an increase in cellularity was found. Haemophagocytes with erythrophagocytosis were detected in 67% of the deceased patients. In tissues with low numbers of haemophagocytes or ill-defined haemophagocytes, an increase in iron deposits was frequently seen. Morphological findings were then correlated with several important clinical data, and the HScore (probability of having a reactive hemophagocytic syndrome) was calculated to posthumously confirm the diagnosis of secondary haemophagocytic lymphohistiocytosis. The median duration of disease and the hospitalisation time were lower in patients with haemophagocytosis (n = 10) than in patients without haemophagocytosis (n = 5). In addition, patients with haemophagocytes showed increased inflammatory parameters 2–5 days prior to death, in contrast to patients without haemophagocytes.

Conclusions: Haemophagocytosis is a common finding in the bone marrow of deceased individuals with severe COVID-19, and may indicate fatal severe acute respiratory syndrome coronavirus 2 infections.

Keywords: autopsy, bone marrow, COVID-19, haemophagocytic lymphohistiocytosis, SARS-CoV-2

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly contagious microorganism responsible for the pandemic of coronavirus disease (COVID-19), which is associated with a severe disease course in high-risk patients. The pathophysiology and mechanisms of the immune response in COVID-19 patients have not yet been completely elucidated. Although respiratory symptoms such as dyspnoea or cough are predominant, the virus affects the entire host organism, and has been detected in many
organs by the use of molecular techniques.\textsuperscript{1,2} Especially in patients with severe COVID-19, dysregulation of the immune system, with elevated levels of proinflammatory cytokines, increased numbers of neutrophils, and decreased numbers of lymphocytes, has been reported.\textsuperscript{3} The first therapeutic trials with anti-inflammatory drugs, such as inhibitors of interleukin (IL)-6 or dexamethasone, have been successful.\textsuperscript{4,5} Similar types of immune dysregulation have been described for other previously identified coronaviruses, such as severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and Middle East respiratory syndrome coronavirus (MERS-CoV).\textsuperscript{6} For SARS-CoV-1, direct infection of immune cells and activation of macrophages have also been reported.\textsuperscript{7,8} Marked hyperferritinaemia and very high amounts of IL-6 in COVID-19 are important indicators of uncontrolled macrophage activation, which is considered to be the main pathological mechanism of secondary haemophagocytic lymphohistiocytosis (sHLH).\textsuperscript{9}

Macrophage activation syndrome (MAS) is a term that is often used synonymously with sHLH, and describes a rare condition with uncontrolled activation of cytotoxic T lymphocytes.\textsuperscript{10} This leads to a proinflammatory cytokine storm with high levels of ILs (e.g. IL-1 and IL-6), hyperferritinaemia, and the activation of macrophages with haemophagocytosis.\textsuperscript{11} Clinically, patients are often critically ill and suffer from fever, hepatosplenomegaly, and organ dysfunction.\textsuperscript{10} There are various causes of sHLH, including neoplasms and autoimmune diseases, but the most common causes are viral and other intracellular infections.\textsuperscript{12}

There are many clinical and laboratory parallels between severe COVID-19 and sHLH of non-COVID-19 origin.\textsuperscript{13} According to the sHLH diagnostic guidelines from 2004 and the so-called HScore from 2014, which calculate the probability of having a reactive hemophagocytic syndrome, one important but non-essential criterion (major criterion) in sHLH diagnostics is haemophagocytosis, which is preferentially found in the bone marrow.\textsuperscript{14,15}

In COVID-19 patients, the occurrence of haemophagocytosis in the bone marrow has already been shown; however, its correlation with morphometry and clinical parameters has been poorly investigated so far.\textsuperscript{16} In particular, there is no analysis of haemophagocytosis in relation to the clinical course and sHLH scoring systems. Therefore, we examined the bone marrow of 15 patients who died from severe COVID-19 for haemophagocytosis. The morphological findings were then correlated with the clinical data and the course of COVID-19.

Materials and methods

Autopsies

In a single-centre study, complete autopsies were performed on 15 bodies of patients who had died from severe COVID-19 between 11 April 2020 and 2 June 2020 at the Institute of Forensic Medicine of the University Hospital Jena. All patients had tested positively for SARS-CoV-2 prior to their death. The median time between death and autopsy was 3.5 h (range, 1.3–26 h). The study was approved by the ethical board of Jena University Hospital (registration no. 2020-1773, 5 May 2020). Important clinical data are summarised in Table 1.

Counting haemophagocytes in the bone marrow

During autopsy, samples of the bone marrow from the vertebral column were formalin-fixed and subsequently embedded in paraffin with routine procedures. Approximately 2-μm tissue sections of embedded bone marrow were stained with haematoxylin and eosin (H&E), Prussian blue, and Gomori. Additional slides were stained with antibodies against monocytes and macrophages [anti-CD163 (Novocastra, Newcastle, UK; NCL-CD163), anti-CD68 (Dako, Glostrup, Denmark; M0876), and anti-CD14 (Santa Cruz, Dallas, TX, USA; 58951)], megakaryocytes/pla- telets [anti-CD61 (Dako; M0753)], erythroid cells [anti-CD71 (Zytomed, Berlin, Germany ACI 3110 B)], myeloid cells [anti-CD34 (Dako; IR632), anti-myeloperoxidase (Dako; IR511) anti-lysozyme (Dako; A0099), and anti-CD117 (Dako; A4502)], lymphoid cells [anti-CD3 (Dako; IR503), anti-CD20 (Dako; IR604), and anti-terminal deoxynucleotidyl transferase (TdT) (Dako; IR093)], mast cells [anti-mast cell tryptase (Dako; IR640) and anti-CD117 (Dako; A4502)] and plasma cells [anti-CD138 (Dako; IR642)] by the use of routine protocols on the Omnis machine (Agilent, Santa Clara, CA, USA). For analysis of the H&E-stained tissues, a region of interest (ROI) was defined on the basis of the anti-CD163 immunostaining. From H&E-stained tissues, 10 representative areas from every slide were randomly selected (each 312.55 × 250.61 μm) and imaged with a Zeiss AxioScope 506 (Zen 2.6 software, blue edition; Jena, Germany) at 400-fold magnification. The total area of counted bone marrow for each patient was 0.783 mm². In the images, haemophagocytes were independently and manually counted by two pathologists (J.S. and N.G.) using Fiji.\textsuperscript{17}
Table 1. Summary of clinical data of 15 deceased coronavirus disease 19 patients

| Clinical data | Number of haemophagocytes |   |   |
|---------------|---------------------------|---|---|
|               | 0                         | 1–5| >5 |
| **Clinical features** |                        |   |   |
| Age (years), median (range) | 80 (64–82) | 77 (54–87) | 80 (53–85) |
|                  | n = 5                    | n = 5 | n = 5 |
| Male sex (%)     | 80                       | 80  | 40 |
|                  | n = 5                    | n = 5 | n = 5 |
| Hospitalisation time (days), median (range) | 14 (1–32) | 10 (5–36) | 6 (3–55) |
|                  | n = 5                    | n = 5 | n = 5 |
| ICU time (days), median (range)/% | 10 (0–16)/80 | 7 (0–8)/80 | 3 (0–55)/60 |
|                  | n = 5                    | n = 5 | n = 5 |
| Therapeutic trial* (%) | 40                       | 40  | 20 |
|                  | n = 5                    | n = 5 | n = 5 |
| Blood transfusion (%) | 40                       | 40  | 20 |
|                  | n = 5                    | n = 5 | n = 5 |
| Long-term immunosuppression (%) | 20                       | 0   | 20 |
|                  | n = 5                    | n = 5 | n = 5 |
| PEMC that can cause haemophagocytosis (%) | 20                       | 20  | 20 |
|                  | n = 5                    | n = 5 | n = 5 |
| Fever (%)        | 0                        | 100 | 0 |
|                  | n = 1                    | n = 5 | n = 2 |
| **Hepatosplenomegaly (%)** |                        |   |   |
| Hepatomegaly     | 0                        | 20  | 0 |
|                  | n = 5                    | n = 5 | n = 5 |
| Splenomegaly     | 60                       | 20  | 0 |
|                  | n = 5                    | n = 5 | n = 5 |
| **Laboratory findings, median (range)** |                        |   |   |
| Haemoglobin (mmol/l) | 5.5 (4.6–6.8) | 5.6 (5.2–6.9) | 6.2 (4.3–9.8) |
|                  | n = 5                    | n = 5 | n = 5 |
| Leucocytes (Gpt/l) | 11.8 (4.7–15.6) | 11.8 (2.4–43.3) | 12.1 (8.7–25.7) |
|                  | n = 5                    | n = 5 | n = 5 |
| Platelets (Gpt/l) | 129 (61–378) | 142 (54–391) | 133 (26–353) |
|                  | n = 5                    | n = 5 | n = 5 |
| Fibrinogen (g/l) | 5.6 (5.2–6.0) | 5.2 (1.0–8.9) | 3.75 (2.7–4.8) |
|                  | n = 2                    | n = 3 | n = 2 |
| Ferritin (µg/l) | 2352 (1382–5606) | 3094 (243–24 688) | 1388 (568–4101) |
|                  | n = 4                    | n = 5 | n = 5 |
| Procalcitonin (µg/l) | 1.14 (0.15–13.09) | 4.93 (0.19–8.13) | 3.16 (0.11–23.2) |
|                  | n = 5                    | n = 5 | n = 5 |
| IL-6 (pg/ml)    | 869.5 (487–1252) | 1570 (681–2464) | 487.95 (26.9–949) |
|                  | n = 2                    | n = 3 | n = 2 |
Incorporated erythrocytes were used as the major morphological criterion to identify haemophagocytes. Phagocytosed leukocytes were used as the minor criterion.

Medical records

The duration of disease, duration of intensive care unit (ICU) treatment, hospitalisation time, therapy, pre-existing medical conditions, and laboratory results were gathered from the hospital electronic patient charts. Additionally, chronological temporal progressions of the inflammatory parameters (IL-6, procalcitonin, and ferritin) in the last 7 days prior to death were included in diagrams, if available.

Calculation of the HScore

The HScore was calculated instead of using the haemophagocytic lymphohistiocytosis (HLH) diagnostic guidelines of 2004, because CD8 cell activity and the soluble IL-2 levels were not determined in most patients. The last laboratory data obtained before death and the organ weights measured during the autopsy were used to calculate the HScore. An HScore of >169 was defined as indicating positivity for sHLH (sensitivity, 93%; specificity, 86%).14 By use of the HScore algorithm, the probability of sHLH was calculated for each patient.

Statistical analysis

Patient characteristics are presented with percentages for nominal values and descriptive statistics (median and range) for ratio values. To determine statistical differences between the groups (none, one to five and more than five haemophagocytes per ROI), the median test was used. A P-value of <0.05 was assumed to be statistically significant.

Results

Haemophagocytosis is found in the bone marrow of deceased COVID-19 patients

The bone marrow of all COVID-19 patients (n = 15) sampled during autopsy showed intact tissue architecture with an osseous framework, and fat and trilineal haematopoiesis markers (Figure 1A–F). In all tissues, increases in cellularity were found, including hematopoietic cells, monocytes, and lymphoid cells. In every case, the number of myeloid cells was substantially higher than the number of erythroid cells (Figure 1E), with higher proportions of myelocytes and metamyelocytes. The number of mature neutrophils was always reduced. The megakaryocytes showed frequent small cell clusters with different cytomorphological features, including both microforms and cells with hyperlobulated nuclei (Figure 1F). The haematopoietic cell populations were intermingled with diffusely distributed CD3-expressing lymphocytes (CD3/CD20 ratio of 9:1) and a few plasma cells, but a high number of monocytes/macrophages. Interestingly, the number of mast cells was highly variable. An increase in the number of CD34+/CD117+ cells was never found, but some TdT+ cells were occasionally visible. There was no evidence of tissue fibrosis or an increase in the number of reticulin fibres. Among the haematopoietic and non-haematopoietic cells, haemophagocytes were detectable in H&E-stained tissue slides, and characterised by grouped erythrocytes in the cytoplasm of a vital cell (Figure 1A,B). The morphological findings in H&E-stained tissue were substantiated by those in serial tissue sections immunostained for CD163 (Figure 1B). In tissues with a low number of haemophagocytes (fewer than five cells per ROI) or morphologically ill-defined haemophagocytes, an increase in iron deposits was frequently found (Figure 1C,D).

To quantify haemophagocytosis, haemophagocytes in each ROI were counted by the use of a digitally assisted algorithm of Fiji.17 In the bone marrow of 10

| Clinical data | Number of haemophagocytes | 0 | 1–5 | >5 |
|---------------|----------------------------|---|-----|----|
| ASAT (µmol/s) | 1.38 (0.88–1.62)            | 1.12 (0.71–55.6) | 0.52 (0.4–2.26) |
| n = 4         | n = 3                      | n = 3 |

ASAT, Aspartate aminotransferase; Gpt, Giga particles; ICU, Intensive care unit; IL, Interleukin; PEMC, Pre-existing medical condition.

*Three patients—lopinavir, ritonavir, hydroxychloroquine, and clarithromycin; one patient—lopinavir, ritonavir, and tocilizumab; one patient—ruxolitinib.
of 15 patients, haemophagocytes with erythrophagocytosis were visible on digitalised slides. Whereas in five patients a low number of haemophagocytes per ROI was found (1–5), the number of haemophagocytes per ROI was moderate in three patients (6–10), and high in two other patients (19 and 38). There was a remarkable increase in iron pigment deposits, especially in the bone marrow of patients without haemophagocytosis (three of five patients). The quantified data are summarised in the stacked bar chart in Figure 2.

**PATIENT CHARACTERISTICS**

Essential clinical data and laboratory parameters of the 15 patients are summarised in Table 1. The median age of the patients was 80 years (range, 53–87 years). Sixty-seven per cent of the patients were men and 33% were women. Seventy-three per cent were treated in an ICU, and 33% received specific therapy with antiviral drugs (four patients), tocilizumab (one patient), or ruxolitinib (one patient). There were trends for the hospitalisation time and ICU time to be shorter in patients with haemophagocytes than in patients without haemophagocytes; although there were many overlaps among groups, these differences were not statistically significant. The main cause of death (13 of 15 subjects) was respiratory failure with typical COVID-19 changes in the lungs. In addition, one patient died from acute right-sided heart failure caused by pulmonary embolism, and another patient died from malignant disease (cervical cancer). All
patients had COVID-19-related lung changes, five of 15 had signs of vasculitis, and four of 15 had thromboembolic disease.

In the present study, a high number of bone marrow-located haemophagocytes were found in patient 14, who received blood transfusions 1 and 2 days prior to death. In four patients, pre-existing medical conditions that could cause haemophagocytosis were found, but the numbers of haemophagocytes in the bone marrow of these patients were very low. Three of 10 patients with haemophagocytosis in the bone marrow had a pre-existing medical condition that can be associated with haemophagocytosis: patient 3 had a fungal superinfection in the lung, patient 4 had chronic lymphatic leukaemia with infiltration of solid organs, and patient 14 had received a kidney transplant in the past.

In seven of seven patients, IL-6 levels were elevated, with a median of 949 pg/ml (range, 27–2464 pg/ml). Twelve of 15 patients had elevated procalcitonin levels. The median procalcitonin levels were 1.14 µg/l (range, 0.15–13.09 µg/l) in the patient group without haemophagocytes, 4.43 µg/l (range, 0.19–8.13 µg/l) in the group with one to five haemophagocytes, and 3.16 µg/l (range, 0.11–23.2 µg/l) in the group with more than five haemophagocytes. However, the differences between patients with and without haemophagocytes did not reach statistical significance. Ferritin levels were elevated in 13 of 14 patients, with a median of 2435 µg/l (range, 243–24 688 µg/l) (Table 2).

The temporal progressions of the inflammatory parameters are shown in Figure 3. Patients with haemophagocytes (patients 1, 2, and 14) showed a substantial increase in inflammatory parameters 2–5 days prior to death. Procalcitonin levels were approximately 30-fold to 55-fold of the normal value. Furthermore, in these patients, there was a 100-fold to 5000-fold increase in IL-6 levels and a 19-fold to 220-fold increase in ferritin levels as compared with the respective normal values. Patients without haemophagocytes (patients 11 and 12) showed only a two-fold increase in the procalcitonin level, a 10-fold increase in the IL-6 level, and a three-fold increase in the ferritin level at maximum. One day prior to death and on the day of death, there were also increases in the procalcitonin and IL-6 levels of the patients without haemophagocytes.

**HScore**

The HScore for each patient was calculated with routine procedures, and is shown in Table 2. Patient 2 had a very high HScore of 191, indicating sHLH. However, during the clinical course, this patient received systemic lysis therapy 4 days prior to death.
Table 2. Clinical and laboratory data and HScore for each patient (n = 15)

| Patient ID | PMI (h) | Hospitalisation time (days) | ICU time (days) | Fever (°C) | Concomitant disease* | Therapeutic trial | Blood transfusion | Long-term IS | Haemoglobin (mmol/l) | Leucocytes (Gpt/l) | Platelets (Gpt/l) | Fibrinogen (g/l) | Ferritin (µg/l) | Procalcitonin (µg/l) | IL-6 (pg/ml) | ASAT (µmol/s) | HScore | Probability of having HS (%) |
|------------|---------|-----------------------------|-----------------|------------|----------------------|------------------|------------------|-------------|---------------------|-----------------|-----------------|----------------|----------------|-------------------------|--------------|----------------|---------|-----------------------------|
| 1          | 3.5     | 7                           | 7               | 38.5       | –                    | LRCH             | PRBCs            | No          | 6.3                 | 3.4             | 55              | 5.2           | 3878         | 8.13                    | 681          | 1.12          | 146     | 20.78                        |
| 2          | 2.3     | 5                           | 5               | 39.5       | –                    | LRCH             | FFP, PRBCs       | No          | 5.6                 | 13.6            | 142             | 8.9          | 24688        | 7.12                    | 2464         | 55            | 191     | 81.03                        |
| 3          | 7.5     | 36                          | 8               | >38.5      | –                    | –                | FFP, PRBCs       | No          | 5.3                 | 2.4             | 142             | U            | 2965         | 0.59                    | 2464         | 0.71          | 127     | 7.47                         |
| 4          | 9.5     | 10                          | 8               | 39         | FS                   | –                | PRBCs            | No          | 5.2                 | 5.3             | 188             | U            | 5094         | 4.93                    | 1570         | 0.21          | 160     | 38.5                         |
| 5          | 15      | 8                           | 8               | >38.5      | CLL                  | –                | PRBCs            | No          | 5.5                 | 10.9            | 129             | U            | 5606         | 0.19                    | 1570         | 0.09          | 54      | 0.09                         |
| 6          | 2.3     | 1                           | 0               | 9         | –                    | –                | PRBCs            | No          | 6.9                 | 43.3            | 391             | U            | 243          | 0.15                    | 88           | 0.21          | 68      | 0.21                         |
| 7          | 5       | 1                           | 0               | 9.5       | –                    | -                | PRBCs            | No          | 8.8                 | 11.9            | 61              | U            | 568          | 0.15                    | 128          | 0.03          | 47      | 0.03                         |
| 8          | 6       | 1                           | 0               | 3         | –                    | –                | PRBCs            | No          | 9.8                 | 11.8            | 353             | U            | 792          | 3.16                    | 169          | 0.03          | 35      | 0.03                         |
| 9          | 6       | 9                           | 0               | 6         | –                    | –                | PRBCs            | No          | 8.2                 | 11.2            | 353             | U            | 188          | 0.91                    | 133          | 0.09          | 95      | 0.09                         |
| 10         | 5       | 3                           | 0               | 3.5       | –                    | –                | PRBCs            | No          | 6.2                 | 11.2            | 61              | U            | 568          | 13.09                   | 189          | 0.03          | 42      | 0.03                         |
| 11         | 3.5     | 19                          | 3               | 1.5       | –                    | –                | PRBCs            | No          | 5.4                 | 12.1            | 169             | U            | 72          | 1.01                    | 111          | 0.01          | 50      | 0.01                         |
| 12         | 1.5     | 14                          | 0               | 15        | –                    | –                | PRBCs            | No          | 4.6                 | 15.6            | 133             | U            | 3225         | 23.2                    | 949          | 0.88          | 26      | 0.88                         |
| 13         | 2.3     | 30                          | 10              | 3         | –                    | –                | PRBCs            | No          | 43                  | 11.8            | 111             | U            | 1479         | 14.4                    | 4101         | 0.42          | 26      | 0.42                         |
| 14         | 1.3     | 3                           | 0               | 1.5       | –                    | –                | PRBCs            | No          | 4.3                 | 12.8            | 378             | U            | 1479         | 1.14                    | 4101         | 0.56          | 84      | 0.56                         |
| 15         | 2.3     | 3                           | 0               | 2.3       | –                    | –                | PRBCs            | No          | 6.2                 | 22.6            | 50              | U            | 1905         | 23.2                    | 491          | 2.26          | 84      | 2.26                         |

ASAT, Aspartate aminotransferase; AZA, Azathioprine; CLL, Chronic lymphatic leukaemia; CSA, Cyclosporin A; FFP, Fresh frozen plasma; FS, Fungal superinfection; GPA, Granulomatosis with polyangiitis; Gpt, Giga particles; HS, Haemophagocytosis syndrome; ICU, Intensive care unit; IL, Interleukin; IS, Immunosuppression; KT, Kidney transplantation; LR, Lopinavir and ritonavir; LRCH, Lopinavir, ritonavir, clarithromycin, and hydroxychloroquine; PMI, Postmortem interval (time between death and autopsy); PRBC, Packed red blood cell; RUX, Ruxolitinib; TCZ, Tocilizumab; U, Unknown.

*That can cause haemophagocytosis.
†Systemic lysis therapy 4 days prior to death.
‡Under tocilizumab.
Figure 3. Course of the infection parameters 7 days prior to death (n = 5).
The increase in the fibrinogen level to 1 g/l may therefore also be related to this intervention. Furthermore, one patient had a 38% probability of having sHLH and another had a 21% probability, whereas the majority of patients (12 of 15 patients) had a probability of having sHLH of <10%.

Discussion

In this study, haemophagocytes were found in the bone marrow of 10 of 15 patients (66.67%) who died from severe COVID-19. Three of five patients without haemophagocytosis had conspicuous phagocytes with a high amount of iron. These cells were also detected in seven of 10 patients with haemophagocytosis, and may reflect phagocytes with degraded erythrocytes, as the degradation of phagocytosed erythrocytes can lead to accumulation of iron in the phagocytes. Moreover, iron might overlie the erythrocytes in the macrophage. Prieto-Pérez et al. examined postmortem bone marrow samples of 17 COVID-19 patients, and found hypercellular bone marrow with haemophagocytes in 16 of 17 cases.

In the present study, a similar patient population was examined, with nearly the same median age and the same distribution of sexes. The discrepancy in the number of patients with haemophagocytes could have resulted from the iron storage mentioned above, the small study populations in both studies, and the size of the examined bone marrow area. The exact examined area was not further specified in the aforementioned study.

Goel et al. examined the sensitivity and specificity of bone marrow haemophagocytosis in HLH. Their control group consisted of 20 random bone marrow aspirates, of which 40% also showed at least one cell with haemophagocytosis, but alternative risk factors for haemophagocytosis were not reported. A well-known trigger to induce haemophagocytosis is blood transfusion. In addition, the development of haemophagocytosis has been described in patients who suffer from malignant neoplasia, autoinflammatory diseases such as lupus erythematosus, or non-SARS-CoV-2 infectious diseases.

Bone marrow aspiration as an invasive diagnostic procedure is mostly performed on patients with haematological diseases, who are also at higher risk of suffering from haemophagocytosis. Therefore, it cannot be ruled out that their control group included some patients with sHLH. They postulated a threshold for haemophagocytes between 0.05% and 0.13% of all nucleated cells in the bone marrow. This resulted in a maximum of 100% in their study. Even if this threshold had been applied to our samples, five of 15 patients could have been diagnosed with haemophagocytosis. Prieto-Pérez et al. did not mention the number of haemophagocytes for each patient.

Even though SARS-CoV-2 is also detectable in the bone marrow, it has not been demonstrated whether haemophagocytosis in COVID-19 patients is caused by direct viral infection of phagocytes or indirectly by a systemic immune response. To elucidate this, histological bone marrow analysis and additional SARS-CoV-2 RNA determination in these bone marrow samples would be required. In this study, we showed a trend for patients with haemophagocytes 2–5 days prior to death to have greater increases in infection parameters, especially IL-6, than patients without haemophagocytes. Therefore, haemophagocytosis could be regarded as a sign of immunological activation in SARS-CoV-2 infections, and this supports the hypothesis that a cytokine storm occurs in some patients with severe COVID-19. In clinical trials, high IL-6 levels correlated with the severity of COVID-19 and the associated mortality. In our study, the median time to death after hospitalisation was lower in patients with haemophagocytosis than in those without haemophagocytosis, suggesting that haemophagocytosis in the bone marrow may be associated with accelerated deterioration. In previous studies, it was shown that sHLH was associated with rapid progression to death in septic patients. Therefore, the association of a severe and lethal disease course with haemophagocytosis may not be COVID-19-specific, particularly as 50% of the patients with haemophagocytosis had other triggers (e.g. blood transfusions, cancer, and other infections).

Although there are MAS-like lesions in COVID-19 patients, only one patient completely fulfilled the HScore criteria for sHLH (6.67% of all patients). This patient received systemic lysis therapy, so a non-sHLH-related increase in the fibrinogen level cannot be ruled out. Most patients in our study did not fulfil the main criteria for sHLH, including pancytopenia, hepatomegaly, and hypofibrinogenaemia. The low number of patients with sHLH corresponds with the clinical results published by Wood et al. Their study identified just three of 40 patients who had an HScore of >169 (7.5% of all patients). A major limitation of the studies of Wood et al. and Prieto-Pérez et al. was that the data needed for calculating the HScore were incomplete. In Prieto-Pérez et al., three living patients had haemophagocytosis findings and fulfilled the HLH-2004 criteria. Again, these patients all had a primary disease that can trigger sHLH. Hence, there
is currently no study available that has examined haemophagocytosis in living patients with slight or severe COVID-19, and that has considered possible other triggers of haemophagocytosis.

The findings of Prieto-Pérez et al. and the data presented here point towards haemophagocytosis being a common finding in the bone marrow of patients who have died from COVID-19. Some of our observations suggest that haemophagocytosis may be associated with rapid deterioration during SARS-CoV-2 infection, and is perhaps preceded by a systemic immune response. This supports the hypothesis that a SARS-CoV-2 infection may lead to a MAS-like disease. This study showed haemophagocytosis and different studies have shown high IL-6 and ferritin levels in patients with severe COVID-19. In the absence of a control group of patients with infections other than SARS-CoV-2, we cannot confirm that haemophagocytosis is caused by SARS-CoV-2. Considering these findings, it is likely that no patient suffered from sHLH. Additional studies that include complete datasets with which to assess the sHLH criteria are needed to investigate this further.

Acknowledgements
We would like to thanks Lisa J. Kahl (Columbia University Department of Biological Science, New York) for writing assistance. Open access funding enabled and organized by ProjektDEAL.

Conflict of interest
The authors declare that they have no conflicts of interest.

Author contributions
J. Swoboda conceived and designed the study, contributed to sample collection, analysed and interpreted the data, and wrote the first draft of the manuscript. D. Wittschieber conceived and designed the study, and contributed to sample collection and writing of the manuscript. J. Sanft, S. Kleemann, S. Elschner and H. Ihle contributed to sample collection and writing of the manuscript. M. Hubig contributed to the statistical analyses. M. Pletz conceived and designed the study, and contributed to sample collection and writing of the manuscript. N. Gassler conceived and designed the study, analysed and interpreted the data, and wrote the first draft manuscript.

References
1. Sekulic M, Harper H, Nezami BG et al. Molecular detection of SARS-CoV-2 infection in FFPE samples and histopathologic findings in fatal SARS-CoV-2 cases. Am. J. Clin. Pathol. 2020; 154; 190–200.
2. Deinhardt-Emmer S, Wittschieber D, Sanft J et al. Early post-mortem mapping of SARS-CoV-2 RNA in patients with COVID-19 and correlation to tissue damage. bioRxiv 2020; 182550. Available at: https://www.biorxiv.org/content/10.1101/2020.07.01.182550v1
3. Qin C, Zhou L, Hu Z et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin. Infect. Dis. 2020; 71: 762–768.
4. Toniati P, Piva S, Cattalini M et al. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: a single center study of 100 patients in Brescia, Italy. Autoimmun. Rev. 2020; 19: 102568.
5. The Recovery Collaborative Group, Horby P, Lim WS et al. Dexamethasone in hospitalized patients with Covid-19—preliminary report. N. Engl. J. Med. 2020. E-pub ahead of print, 14 August.
6. Perlman S, Dandekar AA. Immunopathogenesis of coronavirus infections: implications for SARS. Nat. Rev. Immunol. 2005; 5: 917–927.
7. Page C, Goicochea L, Matthews K et al. Induction of alternatively activated macrophages enhances pathogenesis during severe acute respiratory syndrome coronavirus infection. J. Virol. 2012; 86: 13334–13349.
8. Gu J, Gong E, Zhang B et al. Multiple organ infection and the pathogenesis of SARS. J. Exp. Med. 2005; 202: 415–424.
9. McGonagle D, Sharif K, O’Regan A, Bridgewood C. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. Autoimmun. Rev. 2020; 19: 102537.
10. Al-Samkari H, Berliner N. Hemophagocytic lymphohistiocytosis. Annu. Rev. Pathol. 2018; 13: 27–49.
11. Crayne CB, Albeituni S, Nichols KE, Cron RQ. The immunology of macrophage activation syndrome. Front. Immunol. 2019; 10: 119.
12. Ramos-Casals M, Brito-Zerón P, López-Guillermo A, Khamesha MA, Bosch X. Adult haemophagocytic syndrome. Lancet 2014; 383: 1503–1516.
13. Colafrancesco S, Alessandri C, Conti F, Priori R. COVID-19 gone bad: a new character in the spectrum of the hyperferritinemic syndrome? Autoimmun. Rev. 2020; 19: 102573.
14. Fardet L, Galicier L, Lambotte O et al. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheumatol. 2014; 66: 2613–2620.
15. Henter JI, Horne A, Arico M et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr. Blood Cancer 2007; 48: 124–131.
16. Prieto-Pérez L, Fortes J, Soto C et al. Histiocytic hyperplasia with hemophagocytosis and acute alveolar damage in COVID-19 infection. Mod. Pathol. 2020; 33: 2139–2146.
17. Schindelin J, Arganda-Carreras I, Frise E et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods 2012; 9: 676–682.

18. Alam MZ, Devalaraja S, Haldar M. The heme connection: linking erythrocytes and macrophage biology. Front. Immunol. 2017; 8: 33.

19. Goel S, Polski JM, Imran H. Sensitivity and specificity of bone marrow hemophagocytosis in hemophagocytic lymphohistiocytosis. Ann. Clin. Lab. Sci. 2012; 42: 21–25.

20. McGinnis E, Medvedev N, Richards MJ, Chen LYC, Wong MP. Post-transfusion hemophagocytosis without hemophagocytic lymphohistiocytosis. Mayo Clin. Proc. Innov. Qual. Outcomes 2019; 3: 517–522.

21. Suster S, Hilsenbeck S, Rywlin AM. Reactive histiocytic hyperplasia with hemophagocytosis in hematopoietic organs: a reevaluation of the benign hemophagocytic proliferations. Hum. Pathol. 1988; 19: 705–712.

22. Hirano T, Murakami M. COVID-19: a new virus, but a familiar receptor and cytokine release syndrome. Immunity 2020; 52: 731–733.

23. Sun Y, Dong Y, Wang I et al. Characteristics and prognostic factors of disease severity in patients with COVID-19: the Beijing experience. J. Autoimmun. 2020; 112: 102473.

24. Zhou F, Yu T, Du R et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395: 1054–1062.

25. Kyriazopoulou E, Leventogiannis K, Norrby-Teglund A et al. Macrophage activation-like syndrome: an immunological entity associated with rapid progression to death in sepsis. BMC Med. 2017; 15: 172.

26. Wood H, Jones J, Hui K et al. Secondary HLH is uncommon in severe COVID-19. Br. J. Haematol. 2020; 190: e283–e285.