Title: Cytokine release syndrome is not usually caused by secondary hemophagocytic lymphohistiocytosis in a cohort of 19 critically ill COVID-19 patients

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Severe COVID-19 associated respiratory failure, poses the one challenge of our days. Assessment and treatment of COVID-19 associated hyperinflammation may be key to improve outcomes. It was speculated that in subgroups of patients secondary hemophagocytic lymphohistiocytosis (sHLH) or cytokine release syndrome (CRS) with features of macrophage activation syndrome might drive severe disease trajectories. If confirmed, profound immunosuppressive therapy would be a rationale treatment approach. Over a median observation period of 11 (IQR: 8; 16) days, 19 consecutive confirmed severe COVID-19 patients admitted to our intensive-care-unit were tested for presence of sHLH by two independent experts. HScores and 2004-HLH diagnostic criteria were assessed. Patients were grouped according to short-term clinical courses: discharge from ICU versus ongoing ARDS or death at time of analysis. The median HScore at admission was 157 (IQR: 98;180), without the key clinical triad of HLH, i.e. progressive cytopenia, persistent fever and organomegaly. Independent expert chart review revealed the absence of sHLH in all cases. No patient reached more than 3/6 of modified HLH 2004 criteria. Nevertheless, patients presented hyperinflammation with peripheral neutrophilic signatures (neutrophil/lymphocyte-ratio > 3.5). The latter best paralleled their short-term clinical courses, with declining relative neutrophil numbers prior to extubation (4.4, [IQR: 2.5;6.3]; n = 8) versus those with unfavourable courses (7.6, [IQR: 5.2;31], n = 9). Our study rules out virus induced sHLH as the leading cause of most severe-COVID-19 trajectories. Instead, an associated innate neutrophilic hyperinflammatory response or virus-associated-CRS appears dominant in patients with an unfavourable clinical course. Therapeutic implications are discussed.

The SARS-CoV-2 globally poses medical and economic challenges. While epidemiologists and governments are buying time and capacity through various strategies, it is up to clinicians to optimize the treatment of severe COVID-191,2. Various anti-viral drugs, including the polymerase-, or protease-inhibitors and passive immunization attempts are currently undergoing clinical trial3,4.

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Yet, in cases of serious COVID-19 with pneumonia and acute respiratory distress syndrome (ARDS), tocilizumab, a humanized monoclonal Interleukin-6-receptor antagonist, is administered in a phase II open label clinical trial (NCT04317092) to supress hyperinflammation that is assumed to cause fatal lung and multiorgan injury. This is supported by cohort data from China, which report increased ferritin, and elevated TNF-α, IL-6 and IFN-γ levels in severe versus mild COVID-19 cases. Based on these preliminary data, cytokine release syndrome (CRS) or virus induced adult secondary hemophagocytic lymphohistocytosis (sHLH) have been proposed as underlying aetiology of severe COVID-19. The latter is a clinical syndrome, characterized by massive systemic inflammation, persistent fever flares, hepatosplenomegaly, and severe cytopenia, which is rooted in hemophagocytic activity in bone marrow. It is a rare and often fatal clinical syndrome which can be observed in the context of malignancy, systemic autoimmunity, and viral infections such as Epstein-Barr virus. Full blown sHLH in the latter context usually requires cytotoxic agents, i.e. etoposide, which is not to be used carelessly.

The term CRS originally defines drug toxicity, which can be observed subsequently to monoclonal antibody or adoptive T-cell therapies. The syndrome is rooted in massive aberrant B-, T-cell and monocyte activation and a systemic “cytokine storm”. The clinical course is pleiotropic, with fever, skin rash, cytopenia, neurologic symptoms, coagulopathy, hypotension and eventually organ failure i.e. ARDS. Application of tocilizumab usually resolves symptoms. In the sense of an exuberant anti-viral innate immune response, elevated cytokine levels, fever and CRS-like symptoms, including organ injury have been reported in the context of viral infections, such as H5N1, SARS-CoV-1 and SARS-CoV-2. To avoid confusion, we will refer to this with the term “virus-associated CRS”.

Whether “virus-associated” CRS, sHLH or both are present in severe COVID-19 profoundly impacts our understanding of the disease and impacts on therapeutic strategies. We therefore screened all consecutive 19 COVID-19 patients, who were admitted to our intensive care unit (ICU) for (a) CRS and (b) presence of sHLH, using the HScore, and 2004-HLH-diagnostic-criteria. Charts were reviewed by two independent HLH-experienced clinicians to rule in or out actual sHLH.

Results
Baseline characteristics, treatment and clinical course of severe COVID-19-cases. Nineteen consecutive laboratory confirmed COVID-19-patients with typical chest X-ray (n = 1) or CT-scan (n = 18) were admitted to our ICU. Overall, they were mostly male (89%), with a median age of 70 (IQR: 58; 78) years. All had at least one pre-existing comorbidity or history of organ failure, with arterial hypertension (58%) being the most frequent comorbidity.

Median time from hospital admission to ICU-transfer, time from ICU-admission to oral intubation (n = 17) and median time of observation were 3 (IQR: 0;4), 0 (IQR: 0;0), 11 (IQR: 8;16) days, respectively. Median SOFA-Score and worst Horoviz index were 5 (IQR: 3;7) and 180 (IQR: 125;200) mmHg, respectively. Additional incidence of organ failure other than pulmonary can be retrieved from Table 1.

Patients were treated in accordance with the “surviving sepsis campaign guidelines for severe COVID-19”. All patients received intravenous broad-spectrum antibiotics and regular surveillance for superinfection. Two patients additionally received remdesivir within clinical trials. Most patients (89%) showed rapid deterioration of oxygenation and were intubated after a median of 0 (IQR: 0;0) days.

Our approach resulted in clinical improvement of 47% of patients. After a median of 8.5 (IQR 8;12.5) days, eight patients were extubated, one patient was discharged without need for mechanical ventilation. The remaining cases were judged as unfavourable, due to prolonged weaning, progressive ARDS requiring organ support (n = 7), or death (n = 3) (see Fig. 1). No group differences (favourable versus unfavourable group) were detected with respect to time of observation, comorbidities, SOFA-Score and worst Horovitz index were 5 (IQR: 3;7) versus 4 (IQR: 3;6), p = 0.34) or initial noradrenalin dose (1150 (IQR: 0;2000) versus 600 (IQR: 200;1100) µg/h, p = 0.77). Unfavourable clinical courses were associated with older age (75 (IQR: 60;83) versus 59 (IQR: 3;63) years, p = 0.049) and were observed among both men and women. Yet, higher Horovitz index (200 (IQR: 165;200) versus 150 (IQR: 74;200) mmHg, p = 0.04) was recorded in the “favourable group” during the observation period. Kidney injury (70% versus 22%, p = 0.04), liver failure (60% versus 0%, p = 0.01) and need for organ support were more likely in those who took an unfavourable clinical course (60% versus 11%, p = 0.03). Of note, requirement of organ support did not per se preclude a favourable course (Fig. 1 - ID-6). For more details see Table 1 and Fig. 1.

Immunologic features and relation to clinical course in severe COVID-19. Most patients showed an hyperinflammatory immune response while the pulmonary gas exchange deteriorated. At ICU-admission, most had fever (38.2 (IQR: 37.6;39)°C), all presented with increased C-reactive-protein-, elevated IL-6-, serum ferritin- and sIL-2R- with relatively low procalcitonin-levels (CRP: 20.4 (IQR: 14.4;26.3) mg/dl; IL-6: 139 (IQR: 77;238) mg/dl; sIL-2R: 2140 (IQR: 1097;2843) IU/ml; PCT: 0.8 (IQR: 0.3;3.3) mg/ml, respectively). All patients showed hyper-fibrinogenemia (777 (IQR: 654;859) mg/dl). Splenomegaly (21%) and hepatomegaly (26%) were found in the minority of patients (Table 2).

Whereas fever was volatile within the first week (temperature day 1: 38.2 (IQR: 37.6;39), day 8: 38.7 (IQR 37.3; 38.2)°C, p = 0.20), all patients maintained elevated D-Dimers (day 1: 2961 (IQR: 1498;6548); day 8: 6492 (IQR: 3128;9618) ng/ml, p = 0.37), elevated CRP (day 1: 20.4 (IQR: 14.4;26.3), day 8: 14.3 (IQR: 8;17.3) mg/dl, p = 0.14) and IL-6 levels (day 1: 139 (IQR: 85;872), day 8: 68 (IQR: 38.5;615.9) µg/ml, p = 0.34). Of eight cases who developed significantly elevated PCT ≥ 1 µg/L within the first week, 6 (75%) had positive blood culture or tracheal specimen testing for bacterial superinfection.

On the first day post intubation, most patients required rather high noradrenaline doses (day: 600 (IQR: 0;2000) µg/h). Yet, after a week post ICU-admission, they rarely needed circulatory support (day: 8: 0 (IQR: 0;400),
Cumulatively, these data indicate that the circulatory and pyrogenic impact of the cytokine release associated with COVID-19 was transient and often self-limiting (Table 2).

On the cellular level with varying absolute numbers of leukocytes, relative neutrophilia (84 [IQR: 78;90] %), lymphopenia (9 [IQR: 4;15] %), and reduced percentage of monocytes (3 [IQR: 1;5] %) were observed at ICU-admission (Table 2, Fig. 2 a–c, e). Consequently, neutrophil/lymphocyte ratio (NLR) was increased (10 [IQR: 5;21]; Fig. 2d). Immunophenotypic data revealed rather low numbers of circulating CD8 + T-cell subsets with a trend towards lower values in the unfavourable group (supplementary Table 1).

**Table 1.** Cohort characteristics at time of ICU-admission and clinical course. (Sub)groups: “Overall”, “favourable” versus “unfavourable” clinical group. Statistical comparisons were done using ANOVA-, Mann–Whitney- and Kruskal–Wallis-test as appropriate comparing “favourable” versus “unfavourable” clinical groups. *Comorbidities were defined as follows: Pulmonary disease: Asthma, chronic obstructive pulmonary disease or fibrotic lung disease; Cardiovascular disease (CVD): Pulmonary artery embolism, peripheral arterial occlusive disease, history of lung oedema; Atrial fibrillation (AF); Liver disease: history of gastrointestinal bleeding, liver-cirrhosis or pancreatitis;** Ulcerative colitis; ***prior immunosuppression included recent history of stem-cell transplantation, intake of cyclosporine and low dose steroids. Abbreviation: Advanced organ support (ADVOS), acute kidney injury – transient / requiring dialysis (AKI), Aspartate-aminotransferase (AST), chronic kidney disease (CKD), extracorporeal membrane oxygenation (ECMO), not applicable (n.a.), sustained low efficiency dialysis (SLED), Sepsis-related organ failure assessment score (SOFA).

\[ p = 0.05 \]. Cumulatively, these data indicate that the circulatory and pyrogenic impact of the cytokine release associated with COVID-19 was transient and often self-limiting (Table 2).
Strikingly, relative neutrophilia, lymphopenia and NLR paralleled the clinical course of our patients (Fig. 2b-d, Table 2). Of note, these intergroup differences could not be replicated in absolute cell numbers of the respective cell populations, likely due to the pronounced interindividual variation in leukocytes (supplementary Fig. 1). After the first week of intubation, patients in the favourable group separated and showed significantly decreased relative neutrophilia, lymphopenia and NLR the day prior to successful extubation, whereas NLR remained increased in the unfavourable group (favourable: 10.3 [IQR: 3.5;21.3] → 5.2 [IQR: 2.9;12.5] → 4.4 [IQR: 2.5;6.3] versus unfavourable: 7.8 [IQR: 4.3;31.9] → 12.9 [IQR: 4.1;12.9] → 7.6 [IQR: 5.2;31]; p = 0.03; Fig. 2b-d). The decline of CRP over this period was less pronounced and only separated the groups prior to extubation (Fig. 2f). Interestingly, despite a declining trend over the first week, serum IL-6-levels did not separate favourable and unfavourable clinical courses (median at tube removal: 32 [IQR: 13;49], versus 65 [IQR: 52;150] pg/ml, p = 0.15; Fig. 2g). D-Dimers tended to increase in both groups (repeated-measure-ANOVA: p = 0.13 or 0.2, Fig. 2h). Thus, an acute neutrophilic response rather than serum mediator kinetics paralleled the clinical course in our patients. The lack of organomegaly and lymphopenia suggest that hyperinflammation is linked to innate immunity.

Assessment of virus induced HLH. Due to fever, hyperinflammation and high mortality, we asked whether patients with severe COVID-19-disease and organ dysfunction suffer from virus induced secondary HLH.

The median HScore of our patients was 122 (IQR: 63;145) or 157 (IQR: 98;180) if positive bone marrow histology criterion was assumed. Therefore, 6 (32%) patients would have reached the proposed cut-off of 169 for ‘suspicion of hemophagocytosis’. Herein a trend for higher HScores in the unfavourable group (139 [IQR: 95;156] versus 90 [IQR: 63;123.5], p = 0.1) was apparent, with 5/10 (50%) versus 1/9 (11%) patients reaching the cut-off of 169 in the respective groups. Substantial domains of the HScore, i.e. fever, spleno-, hepato-megaly, bicytopenia, were assessed positive in 5 (26%), 4 (21%), 5 (26%), 7 (37%) patients, respectively. None of the patients developed hypofibrinogenemia. Elevations in AST were detected in all patients (Table 3). 8 (42%) developed serum ferritin > 2000, and two exceeded 6000 ng/ml. Similar observations with an overall slightly lower median HScore of 155 [IQR: 98; 166] were made when calculating the HScore after one week post admission. Still 3 patients (30%) of the unfavourable group achieved the predefined cut-off (supplementary Table 2).

Nevertheless, the HScore is not intended for and has never been validated for use in an ICU-setting. Likewise, higher cut-off values for ferritin have been suggested. We therefore applied “modified”—“2004 HLH-diagnostic-criteria”, which are considered positive in the presence of 4/6 of the following criteria: persistent fever, splenomegaly, persistent or progressive cytopenia, ferritin > 10.000 ng/ml, AST > 50 IU/l. As can be retrieved from Table 3 only one patient from the unfavourable group...
Table 2. Short-term trends of immunologic parameters after ICU-admission. Column 2–4: We report relevant clinical immunologic parameters at time of ICU-admission (ADM) and after *1 week (5–8), while patients that required intubation were still on mechanical ventilation. Paired t-tests, or paired Wilcoxon-rank-test, were used to test for intra-individual differences, as applicable (full cohort). Columns 5 and 6: Group differences (favourable versus unfavourable clinical courses) were assessed using ANOVA or Kruskal–Wallis-test. **Serum ferritin and sIL-2R receptor were assayed after a median of 2 days (missing data n = 1) post admission (ADM) and 4 days later – we report peak values. We report median and interquartile range (IQR) or frequencies as counts and percent of total. ANOVA was used for group comparisons: favourable versus unfavourable clinical course. Abbreviations: Interleukin (IL), C-reactive-protein (CRP), neutrophil/lymphocyte-ratio (NLR), soluble Interleukin 2 receptor (sIL-2R).

| Parameter                  | Overall (n = 19) ICU-ADM | Overall (n = 19) 1 week* | p-value (pairwise) | Favourable (n = 9) | Unfavourable Group (ADM) (n = 10) | p-value (indep.) |
|----------------------------|--------------------------|--------------------------|---------------------|-------------------|-------------------------------|-----------------|
| Body Temperature[°C]       | 38.2 (37.6; 39)          | 37.8 (37.3; 38.2)        | 0.20                | 38.5 (37.7; 38.7)   | 38.1 (37.4; 39.7)            | 0.70            |
| Splenomegaly (1 = yes)     | 4 (21%)                  | n.a                      | n.a                 | 2 (22%)            | 2 (20%)                       | 0.97            |
| Hepatomegaly (1 = yes)     | 5 (26%)                  | n.a                      | n.a                 | 2 (22%)            | 3 (30%)                       | 0.78            |
| CRP [mg/dl]                | 20.4 (14.4; 26.3)        | 14.3 (8.1; 27.3)         | 0.14                | 21.3 (18.6; 24.7)   | 17.5 (12.3; 27.9)            | 0.57            |
| IL-6 [pg/ml]               | 139 (85.8; 872)          | 68 (38.5; 615.5)         | 0.379               | 90.2 (64.8; 119.5)  | 568 (161; 2094)              | 0.14            |
| PCT [ng/ml]                | 0.8 (0.3; 3.3)           | 0.4 (0.2; 1.1)           | 0.24                | 0.4 (0.2; 3.1)      | 1.0 (0.5; 3.4)               | 0.81            |
| N (PCT ≥ 1 ng/ml)          | n.a                      | 8 (42%)                  | n.a                 | 1 (11%)            | 7 (70%)                       | 0.18            |
| N (culture positive)       | 6 (32%)                  | n.a                      | n.a                 | 1 (11%)            | 5 (50%)                       | 0.32            |
| D-Dimer [µg/l]             | 2961 (1498; 6548)        | 6492(3128;9618)          | 0.37                | 2961(1728;7689)     | 2493(1428;6410)              | 0.80            |
| Fibrinogen[mg/dl]          | 777 (654; 859)           | 646 (594; 811)           | 0.03                | 775 (722; 835)      | 785 (530; 868)               | 0.39            |
| sIL-2R [IU/ml] **          | 1404(1097;2843)          | n.a                      | n.a                 | 1271(954;2133)     | 2129(161;4359)               | 0.10            |
| Ferritin [ng/ml] **        | 1815(863;3674)           | n.a                      | n.a                 | 919 (810; 1752)    | 3572(819;5343)               | 0.03            |
| Noradrenaline dose [µg/h]  | 600 (0; 2000)            | 0 (0; 400)               | 0.05                | 600 (200; 1100)    | 1150 (0; 2000)               | 0.77            |
| Leucocytes [10⁹/L]         | 10.0 (6.1; 12.3)         | 9.4 (6.8; 12.3)          | 0.90                | 8.4 (5.4; 11.3)    | 10.6 (5.9; 14.0)             | 0.42            |
| Neutrophils (%)            | 84 (78; 90)              | 76 (72; 88)              | 0.01                | 85 (82; 90)        | 84 (77; 92)                  | 0.36            |
| Lymphocytes (%)            | 9 (4; 15)                | 11 (6; 14)               | 0.98                | 8 (4.5; 12.5)      | 13 (4; 17)                   | 0.37            |
| NLR                        | 10 (10; 21)              | 6 (5; 13)                | 0.027               | 11 (7; 20)         | 7 (5; 26)                    | 0.89            |
| Monocytes (%)              | 3 (1; 5)                 | 6 (2; 10)                | 0.03                | 3 (1; 5.5)         | 3 (1; 6)                     | 0.54            |
| Body temperature [°C]      | 38.2 (37.6; 39)          | 37.8 (37.3; 38.2)        | 0.20                | 38.5 (37.7; 38.7)   | 38.1 (37.4; 39.5)            | 0.70            |

reached the modified cut-off for serum ferritin. The sustained fever- and cytopenia-criteria were reached by 3 (16%) and 6 (32%) patients, respectively. 13 (68%) patients showed hypertriglyceridemia (Table 3). Fever and cytopenia were judged in a strictly longitudinal manner. The remaining domains were identical to the HScores-domains (Table 3), this resulted in 0/19 patients fulfilling four of the “modified”—“2004-HLH-diagnostic- criteria”.

In line with this, sHLH usually implies massive activation of T-cellular immunity, which can be assayed via elevated sIL-2R 21. Although 5 (26%) of our patients showed values above 2000 U/ml, none exceeded the limit of 10,000 U/ml, which has high specificity for sHLH.

Most importantly, sHLH is a clinical syndrome, which is ultimately defined by the assessment of the experienced clinician. We therefore had all case-charts (supplementary file 1) reviewed by experts. Both reached agreement, that the diagnostic criteria for classical sHLH were not fulfilled in any of the presented cases. (Table 3). However, CRS-like hyperinflammation associated with COVID-19 was assessed positive in 11 (58%) cases. 8 (42%) patients were defined “uncertain” regarding the latter due to concomitant superinfection (Table 3).

In summary, none of the patients showed evidence for secondary HLH within a median of 11 (8;16) days of observation after ICU-admission, despite the presence of ARDS, systemic hyperinflammation and eventually organ failure. Thus, virus-associated CRS but not sHLH is frequently associated with severe COVID-19.

Discussion

Based on SARS-CoV-1 and preliminary results of recent anti-cytokine-targeted interventional studies, an exuberant immune response has already been extrapolated as a major cause of lung injury, organ failure and mortality in COVID-19 22. In fact, hyperinflammation and hyperferritinemia are hallmarks of severe over moderate COVID-19 23,24. Moreover, hypercoagulability causing thrombosis, a classical feature of secondary HLH, has been observed amongst 30% of severe COVID-19 patients 12. And more recent evidence suggests, that this hyperinflammation can replicate different facets of both sHLH and viral CRS in different patient groups and different timepoints of disease courses 12,23. These observations gave rise to the idea, that “virus associated” CRS or even sHLH might drive severe COVID-19 7,25.

Here we report on the clinical course and immunologic findings of 19 consecutive severe COVID-19-patients. We demonstrate that up to a median of 11 (8;16) days post ICU-admission none of our patients showed classical evidence of sHLH, which practically excludes sHLH as an initial driver of severe COVID-19 in the majority of
cases. We relied on various layers of evidence, including the screening for HLH using the HScore as suggested by Mehta P et al. only recent 7. Apart from high HScores in individual cases, all patients lacked the classical clinical triad, i.e. persistent fever, organomegaly and progressive cytopenia 9,26. In support of our data Wood et al. recently reported, that out of 40 severe COVID-19 cases, only three reached the H-score specific sensitivity cut-off of >169. Of note, these patients lacked classical clinical HLH criteria, i.e. organomegaly 27. As also acknowledged by Wood et al., the HScore must be interpreted with caution in severe COVID-19-patients, since ferritin, fever, cytopenia and even the bone marrow criterion (“hemophagocytosis”) lose specificity in an ICU-setting where by Wood et al., the HScore must be interpreted with caution in severe COVID-19-patients, since ferritin, fever, cytopenia and even the bone marrow criterion (“hemophagocytosis”) lose specificity in an ICU-setting where 

**Figure 2.** Immunologic parameters, neutrophilia and IL-6 in relation to clinical course at time of ICU admission, at day 4–7 (median 6 days)—when patients were still intubated—and prior to extubation if applicable (median 10 days). The median of 10 days differs from the median intubation time of 8.5 days (see main manuscript) since not all patients were intubated within 24 h post admission. Data from the unfavourable group (no extubation was possible) were matched accordingly to achieve equivalent median days post ICU admission.

Abbreviations: C-reactive-protein (CRP), Interleukin-(IL)-6, neutrophile/lymphocyte-ratio (NLR). This analysis includes 17 patients who had undergone intubation (1 patient each from the favourable and the unfavourable group were excluded, see methods section). Each datapoint represents one patient. Statistical significance for independent t-test between groups: n.s. = not significant, * = p < 0.05, **p < 0.001. Statistical significance for repeated measure ANOVA † = not significant, ‡ = p < 0.05, §§ = p < 0.01, §§§ = p < 0.001.
similar clinical assessments. Yet, if immune-modulatory drugs meet our expectations, our observations raise the questions of (a) which (sub)groups should be treated and (b) at what timepoint, to not endanger patients with self-limiting courses by overtreatment.

Methods

Study design and patient characteristics and clinical course. This observational cohort study is a pilot study analysis of the multi-centre register of “COVID-19-register to document cases of secondary hemophagocytic lymphohistiocytosis” NCT04347460. All patients, their families or legal guardians gave written and informed consent. The study was approved by the local ethics committee of the Klinikum rechts der Isar of the Technical University of Munich (Ref. 161/20 S) and accordance with the declaration of Helsinki.

Patients’ characteristics were entered based on interviews with patients or families and the clinic’s medial record system. BMI, Horovitz Index (HI) and sequential-organ-failure-assessment-(SOFA)-Score were calculated on admission as body weight [kg]/height$^2$ [m$^2$], paO2/FiO2 or using the online-calculator https://mdcalc.com/sequential-organ-failure-assessment-sofa-score, respectively. Clinical data were independently reviewed

| Parameter                               | Overall       | Favourable (n = 9) | Unfavourable (n = 10) | p-value |
|-----------------------------------------|---------------|--------------------|-----------------------|---------|
| Parameter                               | Median (IQR) / frequency |                    |                       |         |
| HScore (BM assumed neg.)                | 122 (63; 145) 90 (63; 123.5) | 139 (95; 156) | 0.095                |
| n > 169                                 | 1 (5.3%)      | 0 (0%)             | 1 (10%)              | 0.720   |
| HScore (BM assumed pos.)                | 157 (98; 180) 125 (98; 159) | 174 (130; 191) | 0.095                |
| n > 169                                 | 3 (32%)       | 1 (11%)            | 5 (50%)              | 0.156   |
| Likelihood (%)                          | < 10%—98%     | < 10%—54%          | < 10%—98%            | n.a     |
| Prior IS                                | 2 (11%)       | 2 (22%)            | 0 (0%)               | 0.447   |
| Fever (> 38.4 ºC)                       | 5 (26%)       | 2 (22%)            | 3 (30%)              | 0.780   |
| Splenomegaly                            | 4 (21%)       | 2 (22%)            | 2 (20%)              | 0.968   |
| Hepatomegaly                            | 5 (26%)       | 2 (22%)            | 3 (30%)              | 0.780   |
| Bi-Cytopenia                            | 7 (37%)       | 5 (56%)            | 2 (20%)              | 0.21    |
| Tri-Cytopenia                           | 3 (16%)       | 0 (0%)             | 3 (30%)              | 0.28    |
| TAG > 132.7 [mg/dl]                     | 15 (79%)      | 7 (78%)            | 8 (80%)              | 1       |
| TAG > 354 [mg/dl]                       | 4 (21%)       | 2 (22%)            | 2 (20%)              | 0.968   |
| Fibrinogen > 250 [mg/dl]                | 0 (0%)        | 0 (0%)             | 0 (0%)               | 1       |
| Ferritin > 2000 [ng/ml]                 | 8 (42%)       | 1 (11%)            | 7 (70%)              | 0.028   |
| Ferritin > 6000 [ng/ml]                 | 2 (11%)       | 0 (0%)             | 2 (20%)              | 0.497   |
| AST > 30 U/I*                           | 19 (100%)     | 9 (100%)           | 10 (100%)            | 1       |
| Modified HLH 2004 criteria              | 2 (0; 2)      | 1 (0; 2)           | 2 (1; 3)             | 0.113   |
| n ≥ 4 criteria fulfilled:               | 0 (0%)        | 0 (0%)             | 0 (0%)               | 1       |
| Sustained fever                         | 3 (15.8%)     | 1 (11%)            | 2 (20%)              | 0.780   |
| Sustained cytopenia                     | 6 (32%)       | 1 (11%)            | 5 (50%)              | 0.156   |
| Ferritin > 10.000 [ng/ml]               | 1 (5.3%)      | 0 (0%)             | 1 (10%)              | 0.720   |
| TAG-criterion                           | 13 (68%)      | 5 (56%)            | 8 (80%)              | 0.400   |
| sIL-2R > 2400 < 10.000                  | 5 (26%)       | 1 (11%)            | 4 (40%)              | 0.315   |
| Chart review (yes = 1)                  | 0 (0%)        | 0 (0%)             | 0 (0%)               | 1       |
| Expert 1 – HLH (yes)?                   | 0 (0%)        | 0 (0%)             | 0 (0%)               | 1       |
| Expert 2 – HLH (yes)?                   | 0 (0%)        | 0 (0%)             | 0 (0%)               | 1       |
| CRS-like?                               | 11 (58%)      | 6 (67%)            | 5 (50%)              | 0.475   |
| Undefined                               | 8 (42%)       | 3 (33%)            | 5 (50%)              | 0.475   |

Table 3. Expert ratings, median HScores and modified HLH-2004 criteria fulfilled. The table reports median HScores for assumed negative and positive results for the bone marrow criterion. Groups: “overall”, “favourable” versus “unfavourable” clinical group, statistical comparison was done comparing the latter two groups. Further frequencies of the HSscore subdomains are reported. We do the same for the modified 2004 HLH criteria its subdomains. None of our experts detected evidence for HLH in any of the patients’ charts reviewed. CRS like inflammation was rated, when there was evidence for inflammatory disease in the absence of bacterial coinfection. These cases were classified as undefined. Abbreviations: bone marrow (BM) biopsy; Triglycerides (TAG), Aspartate-amino-transferase (AST), hemophagocytic lymphohistiocytosis (HLH), soluble Interleukin-2 receptor (sIL-2R). *The results were identical, when adjusted laboratory cut offs for hypofibrinogenemia and abnormal AST were used.
for intubation” or “was extubated” at time of analysis versus those who were in need of mechanical ventilation, or in need of extracorporeal lung assist devices, prolonged weaning, or had died at time of analysis.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Scores and assessment for HLH. The HScore was calculated at time of ICU-admission and after 1 week as described by Fardet et al. (see Table 4 and supplementary Table 1, respectively 18). Yet, in an ICU-setting only a cut-off value for ferritin ≥ 10.000 ng/ml demonstrated sufficient sensitivity in these patients 19. The bone marrow biopsy criterion was further shown to have poor specificity for HLH among seriously ill patients 47. Thus, the pediatric 2004-HLH criteria, were modified according to centre specific cut-offs, and a cut-off for ferritin of 10.000 ng/ml was used (“modified 2004-HLH criteria”; Table 4). Chart reviews to determine actual presence of sHLH were independently performed by L.G. M.P. and L.R.P., the latter two having at least ten years of experience in diagnosis and treatment of secondary HLH.

Laboratory work and diagnostic procedures. PCR-testing for SARS-CoV2 was performed from tracheal specimens. 18/19 patients had positive results. One patient with disease specific radiology report 15,16, repeatedly tested negative from tracheal specimens, but demonstrated seroconversion for SARS-CoV-2-IgM and IgG. Routine laboratory parameters, sIL-2-receptor (sIL-2R, missing data n = 1) and lymphocyte phenotyping (missing data n = 1) were assayed by clinical central laboratories. Presence of splenomegaly or hepatomegaly were determined from CT-scans or via sonography. Predefined cut-offs were 14 cm or 16 cm respectively 48–50.

Statistics. Was performed using IBM SPSS Version 23. We report median and interquartile range (IQR) or counts and percent of total (%) as applicable. Group comparisons (favourable versus unfavourable clinical group or overall cohort at ICU admission or after 1 week) were done using ANOVA, Mann–Whitney-U-, Kruskal–Wallis-, repeated measure paired ANOVA or paired-Wilcoxon-rank-test as applicable.

Table 4. HScore criteria as reported by Fardet et al. 18 and “modified 2004-HLH-diagnostic-criteria”. “We used centre specific cut-offs for definition of hypo-fibrinogenaemia, hypertriglyceridemia, leucopenia and neutropenia with respect to the “modified HLH2004 criteria”. We used lower or upper laboratory specific reference ranges to define the adjusted cut-offs. In addition the ferritin criterion was modified within the adjusted 2004 HLH-diagnostic guidelines to a cut off of ≥ 10.000 ng/ml, according to better specificity in ICU-patients 18. The HScore is calculated as a sum of points – see Fardet et al. 18. The sum-score can be transformed into a probability score of HLH – however, this has never been validated for assessment of HLH in an ICU-setting. Abbreviations: Aspartate-aminotransferase (AST), Haemoglobin (Hgb), triglycerides (TAG).

| HScore – Sum of points* | Modified 2004 diagnostic criteria * | Points |
|-------------------------|-----------------------------------|--------|
| Immunosuppression: 18   | n.d                               |        |
| Fever: 0 (strictly < 38.4), 33 (38.4–39.4), or 49 (strictly > 39.4) | Fever ≥ 38.5 °C (if persistent -7d) | +1     |
| Organomegaly: 0 (no), 23 (hepatomegaly or splenomegaly), or 38 (hepa-tomegaly and splenomegaly) | Splenomegaly | +1     |
| Cytopenia (lowest within 7d): | Cytopenia | +1     |
| 0 (one lineage), 24 (two lineages), or 34 (three lineages): | (at least bi-lineage, persistent / progressive within 7d) | |
| Hgb ≤ 92 g/L | Hgb < 90 g/L | |
| Platelets ≤ 110 × 10^9/L | Platelets < 100 × 10^9/L | |
| Leucocytes ≤ 5 × 10^9/L | Neutrophils < 1 × 10^9/L* | |
| Ferritin: 0 (< 2000 ng/ml), 35 (2000–6000 ng/ml), or 50 (> 6000 ng/ml) | Ferritin ≥ 10,000 ng/ml * | +1     |
| TAG [mg/dl]: 0 (< 132.7), 44 (132.7–354), or 64 (> 354) | TAG [mg/dl] ≥ 150 | +1     |
| Fibrinogen [mg/dl]: 0 (< 250) or 30 (≥ 250) | Fibrinogen [mg/dl] < 200 | +1     |
| AST ≥ 30 U/E 19 | n.d | |
| Hemophagocytosis in bone marrow – not assessed (35 points) | Hemophagocytosis in bone marrow – not assessed | n.a |

Received: 23 May 2020; Accepted: 5 October 2020
Published online: 26 October 2020

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Acknowledgements

We thank all patients and their families. Further, special thanks to nurses, doctors and all other clinical staff involved in the treatment of these patients.

Author contributions
GL designed the study, did data collection, data analysis, data interpretation, wrote the manuscript, was involved in treatment of the patients and is part of HLH-registry founders. PM was involved design, data collection, data analysis, preparation of figures, data interpretation, reviewed the manuscript, provided expert judgement and is part of HLH-registry founders. QB did data collection, data analysis and preparation of figures, and critically reviewed the manuscript. PL analyzed the data, critically reviewed the manuscript and provided expert clinical judgment. HS was responsible for laboratory data assessment and analysis. MS did data collection, data analysis and preparation of figures and revised the manuscript. CS was involved in treatment of patients, and is part of HLH-registry founders, provided critical input for the manuscript. UH, RMS, HA did data interpretation and provided critical input for the manuscript. TL and WH are taking care of the patients, are part of HLH-registry founders, provided critical input and reviewed the manuscript. CS is head of the founder of the HLH-registry, was responsible for ethics approval, did data analysis, trial registration, data interpretation and was responsible to obtain informed consent statements. GL and PM contributed equally to this work and share correspondence, WH and CS also contributed equally.

Funding
Open Access funding enabled and organized by Projekt DEAL.

Competing interests
Dr. LaRosée reports personal fees from SOBI, personal fees and other from Novartis, outside the submitted work, none of the other authors has conflict of interest.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-75260-w.

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