Reviewer’s report

Title: MACROPHAGE ACTIVATION-LIKE SYNDROME: AN IMMUNOLOGICAL ENTITY ASSOCIATED WITH RAPID PROGRESSION TO DEATH IN SEPSIS

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Reviewer: Thorsten Brenner

Reviewer's report:

General comments

In the present study 3,417 sepsis patients were identified from a Greece cohort by screening for infection and at least two SIRS criteria (study cohort). A second cohort of 109 patients from a previously published Swedish study with severe sepsis/septic shock was supposed to serve as validation cohort. In a second step the 3,417 and 109 patients were screened for meeting the Sepsis-3 definition and features of macrophage activation-like syndrome (MALS). MALS was diagnosed when the Sepsis-3 criteria were fulfilled and either a "HS score 2014" of more than 151 points (score for diagnosis of reactive hemophagocytic syndrome by Fardet et al.) and/or disseminated intravascular coagulation (DIC) along with hepatobiliary dysfunction (HBD) were present. HBD was defined by the presence of at least two of the following criteria: serum bilirubin > 2.5mg/dl, aspartate aminotransferase at least two times higher than the upper normal limit, and international normalized ratio (INR) higher than 1.5. DIC was defined as the presence of both: an absolute platelet count lower than 100,000/mm³ and activated partial thromboplastin time higher than 45 sec (p.9, l.9-12). The aim of the study was to investigate the frequency of MALS in septic patients as well as the development of a biomarker (ferritin) for MALS diagnosis and prognosis.

Finally, 115 patients (3.4%) were diagnosed with MALS in the study cohort and 68 patients (4.0%) in the validation cohort. The mortality within 10 days was 48.9% and 45.2%, respectively. In the context of a ROC analysis in the study cohort, Figure 3B indicates a specificity of 97.4%, a sensitivity of 29.7%, NPV of 97.8% and PPV of 26.1% for a ferritin level above 4,420ng/ml. The authors hereby conclude that ferritin may serve as a reliable biomarker for early detection of MALS (p.11, l. 51-56). The OR for death after 28 days when ferritin exceeds 4,420ng/ml was 4.07 in the study cohort and 3.75 in the validation cohort. Furthermore, the authors claim a correlation between elevated ferritin and a pro-inflammatory state indicated by IL-18, sCD163 levels and IL-10/TNF-α ratio. Additionally, in 36 patients repeated ferritin measurements after 48h were performed. Survivors after 10 days showed a significant decrease of serum ferritin in contrast to non-survivors (Figure 6A). According to the authors a second ROC analysis promised a reliable prediction of death after 10 days with a sensitivity of more than 90% when serum ferritin decreased greater than 20%. Finally, the OR for early death was
shown to be significantly lower in patients with a decrease of serum ferritin more than 20% within the first 48h (Figure 6C)

Although this investigation tries to provide a solution for a clinically relevant issue (delayed identification of MALS in septic patients resulting in a delayed therapy initiation with a new and promising therapeutic approach with anakinra) there are several important concerns which need to be addressed in connection with the presented manuscript:

Major comments:

• There are relevant weaknesses with regard to statistical analyses of the study as well as ensuing data interpretation:

• Based on the results displayed in figure 3B, the statement that serum ferritin (cutoff 4420ng/ml) may serve as a reliable biomarker for early identification of patients suffering from MALS is misleading. A high specificity >97% permits a good performance in order to rule out MALS, whereas the performance of ferritin for the identification of MALS positive patients is low (with a sensitivity of <30%). Or in other words, the likelihood for false negative test results is high. Thus, the statement "our study shows that ferritin serves as a reliable biomarker……"(p. 11, l. 51-56) is misleading and needs to be revised. In addition, in the same sentence the author falsely stated a sensitivity of higher than 97% (p.11, l.53-56) instead of a specificity higher than 97% (again misleading).

Furthermore, if the displayed numbers in figure 3B (n patients) are correct, the results for sensitivity and PPV as well as specificity and NPV are mixed up. The sensitivity should be 26.1%, PPV 29.7%, specificity 97.8% and NPV 97.4%. But still, this does not alter the statement above.

• In figure 5 the authors show a potential relation between ferritin and proinflammatory cytokines and postulate a correlation by comparing mean cytokine levels of patients with ferritin >4 420ng/ml and ≤4 420ng/ml. Although a correlation seems to be reasonable, I would suggest to perform a standard correlation analysis (Pearson, Spearman).

• But the displayed data is still of clinical relevance. A negative test result (ferritin ≤ 4,420ng/ml) seems to exclude a MALS in nearly every case.
• After critical evaluation of the manuscript, one might wonder about the diagnostic criteria for DIC. The diagnostic criteria used within the presented investigation (p. 9, l. 9-12) - only based on aPTT and platelet count - appears to be rather insufficient and might overestimate the incidence of DIC. A well-established scoring system with high sensitivity and specificity should be used instead (DIC Score of International Society of Thrombosis and Hemostasis (ISTH) or Japanese Association for Acute Medicine (JAAM)).

In contrast to non-survivors on day 10, a significant decrease in serum ferritin is described for survivors in the same period. Additionally, the OR for early death for patients with a decrease of serum ferritin over 20% within 48h is significantly lower. In this context, the interpretation of the second ROC analysis on p. 11, l. 24-29 is confusing. How can a decrease in ferritin greater than 20% predict early death after 10 days with a sensitivity >90%? Do we talk about survival?

• The author showed, that the ferritin levels differed significantly between (MALS) survivors and non-survivors on day one and three (Figure 6A). In this context, an initial analysis of the ferritin levels of sepsis patients with and without MALS would be of great interest.

Minor comments:

• The total numbers (n-patients) on the right side in figure 3B seem to be wrong (287 instead of 101, 3130 instead of 3316).

• Why have IL-6 and IFN\(\gamma\) not been considered for further analysis of the pro-inflammatory state? Especially IFN\(\gamma\) levels might be of relevance. There is evidence that IFN\(\gamma\) plays a critical role in MAS pathogenesis and blockade of IFN\(\gamma\) is discussed as novel MAS therapy. Thus, levels of IFN\(\gamma\) could also be of great interest in MALS.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

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