PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Diagnostic Biomarkers for Adult Hemophagocytic Lymphohistiocytosis in Critically Ill Patients (HEMICU): Prospective Observational Study Protocol
AUTHORS	Lachmann, Gunnar; Knaak, Cornelia; von Haefen, Clarissa; Paeschke, Nadine; Meisel, Christian; Nyvlt, Peter; Schuster, Friederike; Piper, Sophie; Kruppa, Jochen; Vorderwülbecke, Gerald; Balzer, Felix; La Roseé, Paul; Schenk, Thomas; Unterwalder, Nadine; Kölsch, Uwe; Lachmann, Nils; Akyüz, Levent; Brunkhorst, Frank; Volk, Hans-Dieter; Keh, Didier; Spies, Claudia

VERSION 1 – REVIEW

REVIEWER	Manisha Madkaikar ICMR-National Institute of Immunohaematology, India
REVIEW RETURNED	12-Jul-2019

GENERAL COMMENTS	It is a well written project covering various aspects of adult HLH. Following points need clarification 1. Concern regarding sample size calculation of 100.: Study includes 16 ICUs in Berlin making only 6-7 patients per ICU to be recruited for the study over a period of 2 years. If the study has to understand prevalence of HLH attempt should be made to include all the patients of suspected HLH in the study. 2. Since in adults, secondary HLH is more common, careful categorization of cases according to predisposing factors and interpretation of biomarkers according to underlying cause will be important. 3. Ideally treatment naïve HLH patients should be included in the study as the study focuses on biomarkers and their diagnostic utility. Some of the biomarkers may change drastically even with few doses of immunosuppressive therapy. Hence data on the immunomodulatory therapy prior to sampling must be recorded and analyzed accordingly. 4. Patients with EBV driven HLH must be evaluated for SAP/XIAP deficiency in addition to other familial HLH. 5. Rather than taking 20ul of antibodies for staining for flowcytometry experiments, titration of individual antibody and taking optimum concentration needs to followed.

REVIEWER	Lauren Henderson Boston Children's Hospital
REVIEW RETURNED	27-Jul-2019

GENERAL COMMENTS	Lachman et al propose to screen patients in 16 ICUs in Berlin for HLH and enroll patients meeting HLH2004 criteria or those

	suspected of having HLH. Enrolled patients with provide biosamples before initiation of treatment. The primary outcome will be the incidence rate of adult HLH among ICU patients. 1) How will the authors ensure they can calculate the incidence of HLH. How will the capture the number of patients admitted to all 16 ICUs. This is not described in the protocol. 2) The inclusion criteria is somewhat unclear. The authors propose to enroll patients meeting HLH 2004 criteria and patients suspected of having HLH. The enrollment criteria for the suspected HLH patients is unclear. Who will decide and by what criteria? 3) How will the authors obtain sufficient controls? In the protocol, they suggest that patients enrolled for suspected HLH but who do not ultimately meet HLH2004 criteria will be controls. Do the authors think there will be sufficient numbers in this group? Won't this group be fairly heterogeneous? 4) The authors could consider measuring CXCL9 as well as the other proposed chemokines.

VERSION 1 – AUTHOR RESPONSE

Referee 1

It is a well written project covering various aspects of adult HLH. Following points need clarification
1. Concern regarding sample size calculation of 100.: Study includes 16 ICUs in Berlin making only 6-7 patients per ICU to be recruited for the study over a period of 2 years. If the study has to understand prevalence of HLH attempt should be made to include all the patients of suspected HLH in the study.

Answer:
Our aim is to recruite all patients with suspected HLH. However, our current experience (the study is ongoing since 09/2018) is that about one third of these patients could not be included for biomarker assessment as the patients or their legal representative declined to participate or a legal representative could not be applied in a timely manner in case of sedated or confused patients. Nevertheless, the number of all patients with suspected HLH from any participating ICU will be documented, irrespective of whether the patient could be recruited to participate in our study. Estimation of adult HLH incidence will refer to all suspected HLH patients at any participating ICU during the study period, not thus to those suspected HLH patients that agreed to participate in our study.
We expanded this to our methods section: “Number of screened patients, number of patients with suspected HLH who could not be included as well as data on all outcome measures will be collected prospectively.”

2. Since in adults, secondary HLH is more common, careful categorization of cases according to predisposing factors and interpretation of biomarkers according to underlying cause will be important.

Answer: We totally agree that the underlying condition and triggers are of major relevance for diagnosis and treatment of adult HLH. Therefore, we expanded the secondary endpoints to trigger and underlying conditions.

3. Ideally treatment naïve HLH patients should be included in the study as the study focuses on biomarkers and their diagnostic utility. Some of the biomarkers may change drastically even with few doses of immunosuppressive therapy. Hence data on the immunomodulatory therapy prior to sampling must be recorded and analyzed accordingly.

Answer: We totally agree that treatment of HLH might have influence on all measured biomarkers. Therefore, we added to the methods section: “If the patient received immunosuppressive therapy prior to inclusion, this will be documented separately.”

4. Patients with EBV driven HLH must be evaluated for SAP/XIAP deficiency in addition to other familial HLH.

Answer: Thank you very much for this important point! As part of the study, all patient give their consent for genetic analyzes of the created biobank in future projects. Unfortunately, it was not planned initially as part of the biomarker analysis within the HEMICU study. However, we recommend this evaluation within the clinical routine to the clinicians in charge when it comes to EBV-HLH.

5. Rather than taking 20ul of antibodies for staining for flowcytometry experiments, titration of individual antibody and taking optimum concentration needs to followed.

Answer: Quantification of HLA-DR on monocytes is performed according to the manufacturer’s instructions (http://www.bdbiosciences.com/ds/is/tds/23-3949.pdf).

Referee 2

Lachman et al propose to screen patients in 16 ICUs in Berlin for HLH and enroll patients meeting HLH2004 criteria or those suspected of having HLH. Enrolled patients with provide biosamples before initiation of treatment. The primary outcome will be the incidence rate of adult HLH among ICU patients.
1) How will the authors ensure they can calculate the incidence of HLH. How will the capture the number of patients admitted to all 16 ICUs. This is not described in the protocol.

Answer: Thank you very much for this important point. All numbers of screened patients as well as patients with suspected HLH who could not be included will be documented. We expanded this to our methods section: “Number of screened patients, number of patients with suspected HLH who could not be included as well as data on all outcome measures will be collected prospectively.”

2) The inclusion criteria is somewhat unclear. The authors propose to enroll patients meeting HLH 2004 criteria and patients suspected of having HLH. The enrollment criteria for the suspected HLH patients is unclear. Who will decide and by what criteria?

Answer: We apologize that screening and recruitment of our study was not described in enough detail. Suspected HLH is assumed when the patient has bicytopenia, hyperferritinemia, and fever, or when HLH is suspected by the clinicians. We specified this in our methods section/inclusion criteria: Suspected or diagnosed HLH: based on HLH-2004 diagnostic criteria (bicytopenia, hyperferritinemia (≥500 µg/L), fever) or suspicion by the clinicians.

3) How will the authors obtain sufficient controls? In the protocol, they suggest that patients enrolled for suspected HLH but who do not ultimately meet HLH2004 criteria will be controls. Do the authors think there will be sufficient numbers in this group? Won't this group be fairly heterogeneous?

Answer: We are confident that our study will include a sufficient number of controls. Indeed, as our study is ongoing since 09/2018, we currently see an HLH rate of about 60% of all included patients. We agree that this group could be heterogeneous, but this might also be a strength to better represent ICU cohorts in clinical routine. Thus, results may be more generalizable.

4) The authors could consider measuring CXCL9 as well as the other proposed chemokines.

Answer: Thank you very much for this important point! In order to the already measured Chemokines CCL2 (MCP-1), CCL3, CCL4, CCL5 (RANTES), CCL11 (Eotaxin), CCL19, CCL20, CXCL1 CXCL8 (IL-8), CXCL10 (IP-10), and CXCL12 (SDF1A), CXCL9 will now also be measured and is, therefore, included in the manuscript.

VERSION 2 – REVIEW

REVIEWER	Manisha Madkaikar NATIONAL INSTITUTE OF IMMUNOHANOHAEMATOLOGY
REVIEW RETURNED	04-Sep-2019

GENERAL COMMENTS	Answers to the comments are satisfactory

REVIEWER	Lauren Henderson Boston Children's Hospital, Division of Immunology
REVIEW RETURNED	09-Sep-2019

GENERAL COMMENTS	The authors have addressed my concerns.