“Exploration of variations in leukocyte morphological parameters (MDW and CPD) using the Beckman Coulter DxH900, based on the infectious condition, inflammatory state and response to corticosteroid therapy in septic patients”

Leukocyte **MO**rphology and **COR**ticosteroids response in **SEP**tic patients

MOCORSEP

NON-HUMAN SUBJECT RESEARCH PROTOCOL

Version No. 2.2 of 16/10/2019

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Signature page
of a non-human subject research protocol

Title:

Exploration of variations in leukocyte morphological parameters (MDW and CPD) using the Beckman Coulter DxH900, based on the infectious condition, inflammatory state and response to corticosteroid therapy in septic patients

Leukocyte **MO**rphology and **COR**ticosteroids response in **SEP**tic patients

Acronym: MOCORSEP

Version No. 2.1 of 25/09/2019

Project code:

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Signature:

The research will be conducted in accordance with the protocol and the legislative and regulatory provisions in force.

TABLE OF CONTENTS

1. SUMMARY

Title *(the title of your study will be made public)*		Exploration of leukocyte morphological parameters (MDW and CPD) using the Beckman Coulter DxH900, based on the infectious condition and corticosteroid therapy in septic patients
Short title/acronym *(the title of your study will be made public)*		Leukocyte MOrphology and CORticosteroids response in SEPtic patients/ MOCORSEP
Project team(s)	Number of teams associated with the study, research or assessment*:	• Recruiter centre: Hôpital Raymond Poincaré Garches team (investigator centre) • Non-recruiter centre: URC HU-PIFO team (Data management) • Non-recruiter centre: Hôpital Saint-Louis Lariboisière Fernand-Widal team (statistics)
	Name, title and function of the coordinating team leader:	• Prof. Martin Rottman
Background of the study, research or assessment		Sepsis is a major public health issue, caused by an organ disorder related to an immune response not controlled by the infection. Several randomised trials have shown that the combination of glucocorticoids and mineralocorticoids improves the survival rate of patients in septic shock. The optimal use of this therapeutic approach requires identifying the pro-inflammatory phase patients likely to respond to corticosteroid therapy. Hyperactivation of immune cells is accompanied by morphological changes likely to be characterised by modern haematology instruments, particularly the DxH900 (Beckman Coulter). The DxH900 is able to use specific technology to determine a significant number of morphological characteristics (CPD or Cell Population Data) for circulating leukocytes. Recently, one of these leukocyte morphological parameters, MDW (Monocyte Distribution Width), has been clinically approved for identifying septic patients or those at risk of developing sepsis. This MDW parameter currently has the CE IVD mark and is approved by the American FDA (7, 8). The aim of the study is to explore the hypothesis that leukocyte morphological parameters, including MDW, may constitute functional biomarkers of pro-inflammatory response and help to identify septic patients likely to optimally respond to a combination treatment of glucocorticoids and mineralocorticoids, and thus improve their chances of survival.
Primary and secondary objectives		Primary objective: 1. Identify whether the leukocyte morphological parameters determined by DxH900 (CPD including MDW) differ between control patients and patients with: • an infection (known or suspected) • sepsis • septic shock - Responders to corticosteroid therapy - Non-responders to corticosteroid therapy Secondary objectives: 1. Identify whether the CPD parameters differ between patients with a pro-inflammatory state for those presenting signs of immunosuppression 2. Explore the relevance of CPD parameters for predicting and monitoring the response to corticosteroid therapy in patients with septic shock.

Specify in a few lines the reason for public interest in the study, research or assessment	Sepsis is a major public health concern in France and worldwide. In France, the mortality of patients with sepsis is 27%, but the mortality of the most serious form (septic shock) can reach 50%. The number of deaths from sepsis in France is estimated to be 30,000. Defining the optimal treatment methods is therefore essential to improving the survival of patients. In particular, use of the mineralocorticoid-glucocorticoid combination has improved the survival rate of only some patients with septic shock (1, 2). The results of the proposed study may lead to identifying a simple test that would select patients likely to respond favourably to corticosteroid therapy. The information regarding leukocyte morphologies are available directly with the results of the complete blood count from the instrument and carried out during a routine blood test. This analysis does not require any additional handling. The costs associated with these morphological tests are practically zero. Considering, on the one hand, the potential impact of these CPD and MDW tests on treatment optimisation and patient survival and, on the other hand, their virtually non-existent costs, therefore, in the event of positive results from this study, we can expect an extremely favourable cost-effectiveness ratio. Therefore, this study presents a major topic of public interest.
Type of study (retrospective cohort, case-control etc.)	Non-interventional, prospective, monocentric study on the exploration of leukocyte morphological parameters according to the infectious condition and response to corticosteroid therapy of septic patients.
Population concerned (inclusion and non-inclusion criteria)	<u>Inclusion criteria:</u> <u>Patients aged at least 18 years</u> Patients in the Intensive Care Unit with: - an infection (known or suspected) - sepsis - sepsis + vasopressor - septic shock • Responders to corticosteroid therapy • Non-responders to corticosteroid therapy <u>Non-inclusion criteria:</u> 1. Pregnant women 2. Patients with onco-haematological diseases with presence of blasts.
Size of the study population	• Size of the population studied: • controls (n=50) • infection (n=50) • sepsis (n=25) • sepsis + vasopressor (25) • septic shock (n=25) • Period covered by the data: 7 days after inclusion <u>Prospective:</u> • Period of data collection: October 2019 to April 2020 (7 months) • Monitoring period: 7 days • Total duration of research: 12 months

Information for participants	Individual or collective information: Collective information in the form of clearly visible flyers will be posted in the Intensive Care Unit and medical and surgical departments of Hôpital Raymond Poincaré, where cases of septic patients, as well as infected patients without sepsis, and control patients will be recruited. An information leaflet will be given to patients upon admission. And the patient's non-opposition statement will be noted in the medical record (ORBIS). For patients who were not able to give their non-opposition statement during their hospitalization, an email will be sent to obtain their non-opposition statement. If no response is received within 3 weeks, the patient will be considered as not being opposed to the use of their data.	
Origin of personal health data (source(s) used)	Personal health data will be extracted from the hospital information system (ORBIS). Laboratory data will be extracted from the hospital laboratory information system (GLIMS). The laboratory data corresponding to leukocyte morphological parameters will be extracted from the DxH900 instrument.	
Personal data collection method (paper, electronic etc.) and database hosting	The data collection method consists of an extraction from the electronic patient record (ORBIS/GLIMS). The clinical and biological data of included patients will be collected electronically by extraction from the hospital computer system (ORBIS). A pseudonymisation step will enable the personally identifiable information of patients (surname, first name, complete date of birth) to not be reported in the CSV file of the study data. The password-protected CSV file will be hosted on a local APHP server at Hôpital Raymond Poincaré, accessible only by authorised profiles via the APHP Intranet (study statistician at Hôpital Saint Louis).	
Pairing method and criteria, if applicable	It is an exploratory study on the leukocyte morphological parameters between patients with various phases of infection and non-infected patients; no pairing with data is planned other than those which will be collected.	
Personal data system and privacy protection method	The data are pseudonymised, stored in a password-protected CSV file, saved in a folder dedicated to the study, and secured by restricting access to participants only. This study folder is stored in a secure folder in the Haematology Department, PARAF (autonomous file sharing on the secure hospital group network) being on the secure APHP server. All of these folders are accessible only by username and password by authorised medical personnel of Hôpital Raymond Poincaré. For the purposes of statistical analysis, the secure data file will be accessible by the URC of Hôpital Saint-Louis Lariboisière Fernand-Widal which will perform the analyses. The database will be exclusively hosted on the secure APHP servers of Hôpital Raymond Poincaré.	
Main variables	Age Sex Body temperature (°C) Heart rate (/min.) Respiratory rate (/min.) Urinary passage (mL/day) QSOFA SOFA	WBC (x109/L) HgB (g/dL) Platelets (x109/L) Neutrophil % Lymphocyte % Monocyte % Eosinophil % Neutrophil (x109/L) MDW

	Vasopressor – single Vasopressor – multiple 43 leukocyte morphological variables from DxH900	PCT (mg/L) CRP (mg/L) IL-6 (pg/L) IFNγ (pg/L) IL-10 (pg/L) Glucose (mmol/L) Urea (mmol/L) Creatinine (µmol/L) Total bilirubin (umol/L) Lactate (mmol/L) AST (IU/L) ALT (IU/L) INR pH pCO2 pO2 HCO3 O2 Sat%
Data analysis method	Descriptive and comparative analyses with modelling of the correlation between data over time and analysis of predictive accuracy by means of linear models and definition of the ROC curve and AUC. Each parameter of CPD morphology measured at a specific time will first be compared between groups (control, patients suspected of infection, patients with septicaemia, patients with septicaemia dependent on vasopressor and lactate treatment < 2, and those in septic shock). Then, they will be compared with patients having a pro-inflammatory profile and those having an immunocompromised profile. Finally, they will be compared with responders and non-responders to corticosteroid therapy. The predictive accuracy based on these parameters will also be estimated. First, to quantify the discrimination capacity of these parameters in the distinctive groups, ROC curves will be plotted, with the C-index as a measure of discrimination. The Hosmer-Lemeshow statistical analysis will be used to measure the calibration. In order to take into account the correlation between the measured data over time on the same subjects, generalised linear models along with random intercepts and slope determination will be used.	
Schedule and organisation of the study, research or assessment	Start of study: 1 October 2019 End of inclusion: 30 April 2020 Set-up of final database: 30 May 2020 Data analysis: 30 June 2020 Study results and report: September 2020	

2. ABBREVIATIONS LIST

CPD: “Cell Population Data” – Leukocyte morphological data
MDW: “Monocyte Distribution Width” – Monocyte size dispersion data
ESId: “Early Sepsis Indicator” – Early indicator of sepsis or risk of sepsis
SOFA: “Sequential Organ Failure Assessment” – SOFA score
QSOFA: “Quick SOFA” – QSOFA score

3. SCIENTIFIC GROUNDS FOR THE RESEARCH

3.1. CURRENT KNOWLEDGE RELATING TO THE AREA CONCERNED

Sepsis is a major public health issue and one of the main causes of mortality and morbidity, with an annual prevalence estimated at 31.5 million and an annual death rate of 5.3 million worldwide (3, 4).

Sepsis is defined as a life-threatening organ dysfunction related to an uncontrolled response of the host to the infection (5). Clinically, sepsis is defined according to an international consensus by using the SOFA (Sequential Organ Failure Assessment) or QSOFA (Quick SOFA) score (5). In a patient presenting a potential infection, a SOFA score > 2 indicates a sepsis-related mortality risk increased by 10%. Septic shock, the most severe form of sepsis, is associated with the highest mortality rate in the order of 40%. Septic shock is identifiable clinically by the need to use a vasopressor treatment to maintain blood pressure at 65 mmHg and a serum lactate concentration > 2 mmol/L (>18 mg/dL) in the absence of hypovolaemia (5).

3.1.1. Corticosteroids and treatment of sepsis

Sepsis results from a host's uncontrolled response to a pathogenic infection. This pathophysiological response can be characterised by a rapid massive systemic inflammation or long-term paralysis of the immune system causing severe recurrent infections and an increase in associated mortality (6).

The treatment is not specific and is based on controlling the source of infection and inflammation and supporting the failing organs. In patients with septic shock, two large multicentre trials, undertaken 15 years apart, showed that the combination of hydrocortisone and fludrocortisone significantly reduces mortality (1, 2). The absolute reduction of mortality in these two trials was approximately 6.5%.

It is likely that among the heterogeneous population of septic patients, some patients may derive a substantial benefit in terms of survival while others do not respond optimally to the corticosteroid therapy. There is a wide consensus on the need to identify patients with a sensitivity to corticosteroids who are likely to benefit from such a therapeutic approach, versus those resistant to corticosteroids for whom this type of treatment is unnecessary or even harmful.

3.1.2. Leukocyte morphologies and sepsis

In response to the infection, neutrophils and the activation of monocytes lead to morphological changes in cell volume and alterations in intracellular structure reflecting the early secretions of cytokines, or late stage cellular events such as diapedesis.

The new DxH900 haematology analyser (Beckman Coulter) uses VCS technology (volume, conductivity, and scatter) with electrical impedance, radio frequency conductivity, and light diffusion to define up to 43 different leukocyte morphological parameters ("cell population data" or CPD).

Recent evidence suggests that the volume and variability of neutrophils and monocytes increase among infected or septic patients versus non-infected donors or those with systemic inflammation (7). A recent multicentre clinical study validated one of these parameters for monocyte volume distribution width (MDW) (referred to as "Early Sepsis Indicator" or "ESId") as an early indicator of septicaemia or development of septicaemia in the context of emergency departments (ED) (8). The MDW parameter is a CE IVD mark test and approved by the American FDA to aid in identifying septic patients or those at risk of developing sepsis (IFU C21894AA).

The CPD and MDW parameters indicate the presence of an infection or sepsis, but also the severity of the pathology (7, 8).

3.2. DESCRIPTION OF THE ELEMENT(S) ON WHICH THE RESEARCH IS BASED

The proposed study seeks to identify whether the MDW or CPD morphological parameters measured on the DxH900 instrument vary according to the different infectious conditions of patients ranging from a known or suspected infection to septic shock versus non-infected control patients, and possibly based on the response to corticosteroid therapy.

3.3. EXPECTED OUTCOMES AND PERSPECTIVES/HYPOTHESES/REASON FOR PUBLIC INTEREST

The hypothesis formulated is that MDW and/or other CPD parameters could be used as functional biomarkers reflecting the progression of sepsis and could be useful in identifying septic patients with a favourable profile who could derive maximum benefit from corticosteroid therapy.

In the event of positive results, this study could pave the way to validation of MDW or other CPD parameters as predictive testing or monitoring of the response to corticosteroid therapy.

Optimisation of a therapeutic approach utilising corticosteroids with functional biomarkers, such as CPD, presents a clear interest in terms of potential improvement of response to treatment and consequently the survival of patients in septic shock.

4. OBJECTIVES

4.1. PRIMARY OBJECTIVE

- Identify whether the leukocyte morphological parameters determined by DxH900 (CPD including MDW) differ between control patients (non-infected) and patients with:
    - an infection (known or suspected)
    - sepsis
    - septic shock
        - Responders to corticosteroid therapy
        - Non-responders to corticosteroid therapy

4.2. SECONDARY OBJECTIVES

- Identify whether the CPD parameters differ between patients presenting with a pro-inflammatory state for those presenting with signs of immunosuppression;
- Explore the relevance of CPD parameters for predicting and monitoring the response to corticosteroid therapy in patients with septic shock.

5. METHOD AND POPULATION

5.1. TYPE OF STUDY

The proposed study is a non-interventional, prospective, monocentric cohort study to explore the variation of leukocyte morphological parameters measured on the DxH900 haematology analyser according to the different infectious conditions of patients ranging from a known or suspected infection to septic shock versus non-infected control patients, and possibly based on the response. This observational cohort study will consist of patients with a known or suspected infection, sepsis or in septic shock on their date of admission into one of

the medical or surgical departments and monitored up to day 7 of the hospital stay or on the date of discharge from the hospital, whichever occurs first.

The design of the study does not require any modification of the existing clinical practice or routine care practice for the treatment of these patients. The laboratory analyses will be carried out according to the practices and recommendations of the existing clinical practice. The additional analyses necessary in order to obtain the leukocyte morphology (MDW and CPD) information will be carried out on a DxH900 instrument from standard laboratory samples and will be carried out based on the analyses made on the haematology instruments routinely used in the laboratory.

Therefore, the design of this study will not require any additional blood sampling other than that already requested as part of standard care.

5.2. STUDY PERIOD

According to the prevalence of sepsis observed at Hôpital Raymond Poincaré and the number of patients to be recruited, the estimated duration of data collection would be approximately 8 months and the total study period should be 12 months from the start date.

5.3. SOURCES OF PERSONAL DATA

Clinical and biological data as well as laboratory information will be extracted from the hospital information system for all patients aged 18 years and older and admitted for a full hospitalisation in one of the medical or surgical departments of Hôpital Raymond Poincaré in order to create a database. Each category of patients participating in the study will be identified according to a defined diagnostic algorithm (see 5.4).

A pseudonymisation step will enable the personally identifiable information of patients to not be reported in the database.

The electronic database created will be hosted on the secure servers of the hospital and administered by the URC of Hôpital Raymond Poincaré.

For the purposes of statistical analysis, the pseudonymised database may be made available to the URC statistician of Hôpital Saint-Louis Lariboisière Fernand-Widal via the APHP Intranet (access with a login and password to the study folder on the PARAF server).

5.4. POPULATION STUDIED AND ENDPOINTS

The population constituting the primary database in this study will be the adult patient population (aged 18 years and over) hospitalised in the medical or surgical departments of Hôpital Raymond Poincaré between 1 October 2019 and 30 April 2020. From this population, the different categories of patients targeted in this study will be identified by means of standard diagnostic criteria, namely: Infection or suspicion of infection in the absence of sepsis; sepsis; sepsis and vasopressors with lactate ≤ 2 mmol/L; and septic shock.

The criteria explored in this study are leukocyte morphological parameters, namely CPD (Cell Population Data) and mainly MDW (Monocyte Distribution Width).

5.4.1. Case – Septic patients

The diagnostic categories to consider for the case to be included in the study are:

- Based on the severity of the disease
  - Infection, but not sepsis
  - Sepsis + SOFA > 2
  - Sepsis (SOFA > 2) + vasopressors and lactate ≤ 2 mmol/L
  - Septic shock (SOFA > 2 + vasopressor + lactate > 2 mmol/L)
- Based on the immune status
  - Pro-inflammatory profile
  - Non-pro-inflammatory profile
- Based on the response to corticosteroids
  - Responder
  - Non-responders

The cases will be identified in the created database (see 5.3), according to the following diagnosis:

- Infection or suspicion of infection in the absence of sepsis (n = 50).

  The suspicion of infection is defined according to the physician responsible for the patient and who prescribed an antibiotic treatment.

- sepsis (n = 50).

  The diagnosis of sepsis will be based on the definition known as “sepsis-3” by the presence of a suspected or known infection with a documented SOFA score ≥ 2 (5).

- sepsis and vasopressors with lactate ≤ 2 mmol/L (n = 25).

- Septic shock (n = 25).

  Septic shock is defined by sepsis and the need for vasopressors to maintain average blood pressure at 65 mmHg or higher, and having a serum lactate level > 2 mmol/L despite an adequate volume resuscitation (5).

After identifying various patient categories in the database by algorithm programming, the patient data in each of the categories will be reviewed by a medical team, which will confirm the correct patient classification.

Corticosteroid therapy for patients in septic shock consists of a 7-day treatment with a 50 mg hydrocortisone intravenous bolus every 6 hours combined with 50 µg fludrocortisone administered by a gastric tube.

Patients with septic shock will be classified as:

- responders to corticosteroid therapy.
- non-responders to corticosteroid therapy.

The positive clinical response to corticosteroids will be defined on day 7 of treatment, as follows:

1) Patient not deceased
2) Absence of vasopressor treatment
3) Free of mechanical ventilation
4) SOFA score < 6

The pro-inflammatory profile can be defined using the white blood cell count, lymphocyte count, serum concentrations of C-reactive protein and procalcitonin, and serum concentrations of circulating cytokines such as IL-6, IFNγ and IL-10. The IFNγ to IL-10 and IL-6 to IL-10 ratios will be calculated in order to define the pro-inflammatory profile of patients.

Two inflammatory profile categories will be defined:

- Pro-inflammatory profile

- Non-pro-inflammatory profile

5.4.2.Case – Control patients

Patients from the same studied population but without any sign of infection or sepsis will be included as a reference group or control patients (n = 50).

5.5. CONDUCTING THE RESEARCH

All patients aged 18 years and over admitted as part of a full hospitalisation in one of the medical or surgical departments of Hôpital Raymond Poincaré will be informed about the study upon admission. They will receive a printed information leaflet and the possibility to refuse, through their doctor, the use of their healthcare data for the purpose of research. In case of non-opposition, the data from all these patients will be used to create the main database of this research. The blood samples (EDTA) taken as part of the treatment and sent to Haematology will be systematically analysed on the DxH900 to obtain the CPD after having been routinely analysed on the laboratory instruments throughout the monitoring period of the patients included. The number of samples that will be analysed is estimated to be 80 per day. The tubes of these samples will be identified through a specific anonymous number, which will be scanned and saved on the DxH900 with the analysis date. The analyses on the DxH900 must be carried out within three hours following the taking of the blood sample. This specific anonymous number will have a number that corresponds with the identification number of these same samples within the GLIMS hospital software. The correspondence between these 2 numbers will enable all of the samples from a single patient and therefore all of the results of the biological analyses from that same patient to be identified. Finally, GLIMS also contains the PPI number of each patient, which will enable all of the biological results of that same patient to be linked to their ORBIS medical record and identified by the PPI of the patient.

In practice, biological analysis data from the DxH900 and GLIMS will be extracted on a daily basis during the study period, and saved in the dedicated secure space on PARAF in the Haematology Department. Every week, a URC data manager will use these extractions to create links between the patient data from the DxH900 and GLIMS, by using the specific anonymous number. They will also perform the extraction of clinical data from each patient whose data has been linked, from ORBIS through the PPI number present in the data from GLIMS. They will complete the data chaining of biological analyses with clinical data extracted from ORBIS.

Each week, the DxH900 data will be backed up and stored on the laboratory computer system.

The biological analysis data targeted in this study will be the analyses carried out for included case-patients on D0 (inclusion day), D3 and D7 (or discharge day). For control patients, the results of the haematological parameters from the first and only analysis will be used.

6. STATISTICAL ASPECTS

6.1. STATISTICAL JUSTIFICATION OF THE SAMPLE SIZE

As it is an exploratory study, no formal calculation of sample size is performed. Recruitment will continue up to the inclusion of 50 non-infected control patients, 50 patients suspected of infection, 25 patients with sepsis, 25 with sepsis and vasopressors and lactate < 2 mmol/L, and 25 patients in septic shock.

6.2. DESCRIPTION OF STATISTICAL METHODS

6.2.1. Descriptive analysis

Continuous parameters will be expressed as an average, median, standard deviation and interquartile range for the entire population and for each category separately. Categorical variables will be expressed as a number and percentage for the entire population and for each category separately.

The distribution of variables will be compared between the clinical categories and between responders and non-responders to corticosteroid therapy using the Wilcoxon non-parametric analysis or Kruskal-Wallis tests for quantitative variables, and Fisher's exact tests for categorical variables. The same tests will compare the variables between patients with pro-inflammatory and immunocompromised profiles, responders and non-responders to corticosteroid therapy and the severity of the infection and septicaemia.

6.2.2. Statistical models

Given the exploratory nature of this study, each end point will be analysed separately.

Each parameter of CPD morphology measured at a specific time will first be compared between groups (control, patients suspected of infection, patients with septicaemia, patients with septicaemia dependent on vasopressor and lactate < 2, and those in septic shock). Then, they will be compared with patients having a pro-inflammatory profile and those having an immunocompromised profile. Finally, they will be compared with responders and non-responders to corticosteroid therapy. These univariate comparisons will be based on statistical tests (Kruskal-Wallis or Fisher's exact test according to the type of variables).

The predictive accuracy based on these parameters will also be estimated. First, to quantify the discrimination capacity of these parameters in the distinctive groups, ROC curves will be plotted, with the C-index (i.e. ROC area under the curve) used as a measure of discrimination. The Hosmer-Lemeshow statistical analysis will be used to measure the calibration.

In order to take into account the correlation between the measured data over time on the same subjects, generalised linear models along with random intercepts and slope determination will be used. The random effects and residual variance structures will be selected by using the selection criterion of the Schwarz BIC model. The adjusted model will be assessed by examining the residues, and whenever it is advisable, the variables will be changed. The models will be adjusted by using a restricted maximum probability method. However, comparisons of the models involving various fixed effects will be based on maximum likelihood estimates, because probability comparisons between the restricted maximum probability adjustments and various fixed effects are not valid. Specific hypotheses concerning fixed effects will be tested, using the Wald test or likelihood-ratio tests.

Missing data will be handled by a multiple imputation approach, after assessment of the missing hypothesis.

All results will be presented as estimates with their 95% confidence interval (CI). A $p < 0.05$ on both sides will be considered as significant.

The analyses will be carried out using the latest version of the R Project for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria).

7. DATA MANAGEMENT

7.1. DATA COLLECTION METHODS

A database in CSV file format will be created containing the clinical information and laboratory analyses. The information will be used for each included patient on the date of inclusion (D0), 3 days after inclusion (D3), and 7 days after inclusion (D7) or at the time of discharge from the hospital, if it occurs before D7.

7.1.1. Clinical data

The personal patient identification number (PPI) will be used for the extraction of clinical information relevant to the study for the hospital information system (ORBIS). Personal data for identifying patients, such as surname, first name and date of birth, will not reported in the database. The pseudonymisation will therefore take place at the time of the clinical database creation.

Data will be extracted electronically by the data manager of the URC and will form a clinical database, which will then be supplemented by data from laboratory analyses.

7.1.2. Laboratory data

In order to add information from laboratory analyses to the database, the PPI number of the clinical database will be used to chain the clinical and biological data for the same patient.

The DxH900 instrument will not be connected to the hospital computer system. The analyses of MDW and CPD leukocyte morphologies may be retrieved using the patient's tube number and sampling date. Data from the DxH900 will be backed up on a weekly basis. Therefore, the included patient's MDW and CPD information may be added to the laboratory database.

The weekly backup of MDW and CPD data from the DxH900 will be stored on a secure server within the Haematology laboratory. Data stored in the DxH900 will be permanently erased from the instrument by the laboratory personnel before the loaned instrument is returned to Beckman Coulter France.

The extraction from GLIMS and consolidation with MDW and CPD information will be carried out by the URC data manager, and will form a laboratory information database.

7.2. DATA SYSTEM AND PRIVACY PROTECTION METHOD

The clinical and laboratory databases (two CSV files) will be saved on a secure server in a shared folder, via the APHP Intranet whose access is secured by unique individual accounts (APHP number and password). Only authorised profiles have access to this server. In other words, the personnel who will perform the exports and imports in this folder, as well as the HU-PIFO URC data manager who will compile and merge the two files and lastly the statistician responsible for the analysis of the Saint Louis URC study.

In no way shall the sponsor have the right to read or write on the raw data.

7.3. RETENTION OF DOCUMENTS AND DATA

The clinical databases and laboratory analyses will be retained in electronic form (CSV format) on an APHP Intranet password-protected server at Hôpital Raymond Poincaré for the duration of the study or 12 months from the start of patient inclusion.

The MDW and CPD leukocyte morphology information will be erased from the DxH900 instrument, as well as from the hard disk of the external storage drive used for regular backups, before the loaned instrument is returned to Beckman Coulter.

8. ETHICAL AND LEGAL ASPECTS

The treatment of patients will not be changed by their participation in the study and no additional blood sample will be necessary to conduct the study. It is research that does not involve human subjects as defined by Law No. 2012-300 of 5 March 2012 and its application by Decree No. 2016-1537 of 16 November 2016.

No compensation will be paid to the patients or the practitioners responsible for recruitment, or to the biologists responsible for analyses essential to the study.

8.1. ROLE OF THE SPONSOR

The sponsor will ensure the necessary funding to properly carry out the study and will establish a collaboration agreement with the investigator's institution.

The sponsor will provide a jointly validated clinical investigation protocol to the investigator.

The sponsor will provide the DxH900 instrument, reagents and consumables as well as the training of personnel involved in the analysis of samples.

The sponsor will monitor the smooth performance of the study placed under the responsibility of the investigator by means of a regular patient recruitment and database formation progress check.

8.2. METHODS OF INFORMING THE POPULATION CONCERNED

In accordance with the legal provisions in force, any preselected patient will first be individually informed of the study objectives and its methodology through the information leaflet hand-delivered by the investigator or qualified person. The information given to the subject will be indicated in their medical record (ORBIS). The collection of the non-opposition statement from the subject will be noted in their medical record by the investigator or qualified person who collects it. We will also announce the conducting of the study by displaying a specific information notice of non-opposition in the departments concerned.

8.3. REGULATORY APPROACHES ON INFORMATICS AND LIBERTY

This study is part of the "Méthodologie de Référence pour les traitements de données à caractère personnel mis en œuvre dans le cadre des recherches dans le domaine de la santé" (Reference methodology for processing personal data used in healthcare research) (MR-004 amended). The sponsor of the research has signed a compliance commitment to this "Reference methodology" with the CNIL.

8.4. REQUEST FOR AN ETHICS COMMITTEE OPINION

According to the provisions of Law No. 2012-300 of 5 March 2012 and its application by Decree No. 2016-1537 of 16 November 2016, the research described in this protocol does not involve human subjects (non-RIHS). The protocol is consistent with reference methodology MR004. The request for an opinion from a Research Ethics Committee (REC) is advisory.

For the sake of quality and to verify that the research is in accordance with ethical standards, and that the rights of the person involved are respected, particularly in terms of information and right to object, the protocol, patient information leaflets, and the type of data collected in the study will be submitted for opinion to the Ethics Committee of the French Intensive Care Society (FICS: https://www.srlf.org/).

8.5. RESPONSIBILITIES OF THE INVESTIGATOR TOWARDS THE MANAGER

The investigator will be responsible for ensuring that the study is properly conducted in accordance with the protocol mutually defined and validated with the sponsor.

The investigator shall regularly inform the sponsor of the progress of the study and allow direct access to the information required for audits and inspections by the persons authorised by the sponsor. The audit may apply to any stages of the study, from protocol development to the publication of results and to the classification of data used or produced as part of the study.

The investigator will have the responsibility of conducting the study in accordance with the best practices of clinical research, quality procedures implemented locally or any other regulatory requirements.

The investigator shall communicate to the sponsor any problems encountered which could jeopardise the timely completion of the study.

8.6. FINAL RESEARCH REPORT

The investigator undertakes to produce a study report including statistical analysis and describing the conclusions obtained in relation to the study objectives.

9. RULES FOR PUBLICATION

By mutual agreement between the sponsor and the investigator, the study results may be published in national or international peer-reviewed scientific journals.

The publications will respect the international guidelines found in “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” (http://www.icmje.org/). The STARD and QUADAS criteria were used for the design of the study and will be used for its execution and publication of its results (9).

The study will be entered in a public clinical trials registry (clinicaltrials.gov) prior to inclusion of the first patient.

10. BIBLIOGRAPHIE

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11. ADDENDA

11.1. LISTE DES VARIABLES

Variables démographiques :

- Sexe
- Age
- Gestation (O/N)

Variables cliniques :

- Information de traitement préexistant
  - Traitement chimio-thérapeutique (O/N)
    - Si oui préciser nature et durée
  - Traitement immunosuppresseur (O/N)
    - Si oui préciser nature et durée

- Information clinique :

	Date/Time J0 DD:MMM:YY (24:00)	**Date/Time J3** DD:MMM:YY (24:00)	**Date/Time J7** DD:MMM:YY (24:00)
Température corporelle (°C)			
Fréquence cardiaque (/min)			
Fréquence respiratoire (/min)			
Passage urinaire (mL/jour)			
QSOFA			
SOFA			
Vasopresseur - unique			
Vasopresseur - multiple			

  - qSOFA
    - Altération mental (Score de Glasgow)
    - Pression artérielle systolique ≤ 100
    - Fréquence respiratoire ≥ 22 / min

- SOFA
  - Fonction respiratoire (PaO2/FiO2, mm Hg)
  - Fonction de coagulation (plaquette x 103/µL)
  - Fonction nerveuse (Score de Galsgow)
  - Fonction hépatique (Bilirubin, mg/dL)
  - Fonction cardiovasculaire (hypotention)
  - Fonction rénale (Créatinine, passage urinaire)

Variables de laboratoire :

	Date/Time J0 DD:MMM:YY (24:00)	**Date/Time J3** DD:MMM:YY (24:00)	**Date/Time J7** DD:MMM:YY (24:00)
WBC (x109/L)			
HgB (g/dL)			
Plaquettes (x 109/L)			
Neutrophile %			
Lymphocyte %			
Monocyte %			
Eosinophile %			
Neutrophile (x 109/L)			
MDW			
PCT (mg/L)			
CRP (mg/L)			
IL6 (pg/L)			
IFNγ (pg/L)			
IL10 (pg/L)			
Glucose (mmol/L)			
Urée (mmol/L)			
Créatinine (µmol/L)			
Bilirubine total (umol/L)			
Lactate (mMol/L)			
AST (IU/L)			
ALT (IU/L)			
INR			
pH			
pCO2			
pO2			
HCO3			
O2 Sat%			

Données de morphologie leucocytaire du DxH900 :

Neutrophils		Lymphocytes		Monocytes	
Mean volume	MN-V-NE	Mean volume	MN-V-LY	Mean volume	MN-V-MO
SD* volume	SD-V-NE	SD volume	SD-V-LY	SD volume	SD-V-MO
Mean conductivity	MN-C-NE	Mean conductivity	MN-C-LY	Mean conductivity	MN-C-MO
SD conductivity	SD-C-NE	SD conductivity	SD-C-LY	SD conductivity	SD-C-MO
Mean median angle light scatter	MN-MALS-NE	Mean median angle light scatter	MN-MALS-LY	Mean median angle light scatter	MN-MALS-MO
SD median angle light scatter	SD-MALS-NE	SD median angle light scatter	SD-MALS-LY	SD median angle light scatter	SD-MALS-MO
Mean upper median angle light scatter	MN-UMALS-NE	Mean upper median angle light scatter	MN-UMALS-LY	Mean upper median angle light scatter	MN-UMALS-MO
SD upper median angle light scatter	SD-UMALS-NE	SD upper median angle light scatter	SD-UMALS-LY	SD upper median ngle light scatter	SD-UMALS-MO
Mean lower median angle light scatter	MN-LMALS-NE	Mean lower median angle light scatter	MN-LMALS-LY	Mean lower median angle light scatter	MN-LMALS-MO
SD lower median angle light scatter	SD-LMALS-NE	SD lower median angle light scatter	SD-LMALS-LY	SD lower median angle light scatter	SD-LMALS-MO
Mean low angle light scatter	MN-LALS-NE	Mean lower angle light scatter	MN-LALS-LY	Mean lower angle light scatter	MN-LALS-MO
SD low angle light scatter	SD-LALS-NE	SD lower angle light scatter	SD-LALS-LY	SD lower angle light scatter	SD-LALS-MO
Mean axial light loss	MN-AL2-NE	Mean axial light loss	MN-AL2-LY	Mean axial light loss	MN-AL2-MO
SD axial light loss	SD-AL2-NE	SD axial light loss	SD-AL2-LY	SD axial light loss	SD-AL2-MO

*SD: standard deviation.

11.2. LISTE DES EQUIPES ASSOCIEES A LA RECHERCHE

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