The REAnimation Low Immune Status Markers (REALISM) project: a protocol for broad characterisation and follow-up of injury-induced immunosuppression in intensive care unit (ICU) critically ill patients

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ABSTRACT

Introduction The host response to septic shock is dynamic and complex. A sepsis-induced immunosuppression phase has recently been acknowledged and linked to bad outcomes and increased healthcare costs. Moreover, a marked suppression of the immune response has also been partially described in patients hospitalized in intensive care unit (ICU) for severe trauma or burns. It has been hypothesized that immune monitoring could enable identification of patients who might most benefit from novel, adjunctive immunostimulating therapies. However, there is currently neither a clear definition for such injury-induced immunosuppression nor a stratification biomarker compatible with clinical constraints.

Methods and analysis We set up a prospective, longitudinal single-centre clinical study to determine the incidence, severity and persistency of innate and adaptive immune alterations in ICU patients. We optimized a workflow to describe and follow the immunoinflammatory status of 550 patients (septic shock, severe trauma/burn and major surgery) during the first 2 months after their initial injury. On each time point, two immune functional tests will be performed to determine whole-blood TNF-α production in response to ex vivo lipopolysaccharide stimulation and the T lymphocyte proliferation in response to phytohaemagglutinin. In addition, a complete immunophenotyping using flow cytometry including monocyte HLA-DR expression and lymphocyte subsets will be obtained. New markers (ie, levels of expression of host mRNA and viral reactivation) will be also evaluated. Reference intervals will be determined from a cohort of 150 age-matched healthy volunteers. This clinical study will provide, for the first time, data describing the immune status of severe ICU patients over time.

Ethics and dissemination Ethical approval has been obtained from the institutional review board (no 69HCL15_0379) and the French National Security agency for drugs and health-related products. Results will be disseminated through presentations at scientific meetings and publications in peer-reviewed journals.

Strengths and limitations of this study

- This is the first prospective study to provide a broad immune status characterisation in a large cohort of intensive care unit (ICU) patients.
- There is a mid-term assessment (D60) of the immune status in ICU patients, which has never been done before.
- Long-term follow-up will not be addressed here and should be examined in future studies.
- New biomarkers of the immune status will be assessed in comparison to standardised tools and immune functional assays.
- Whether such biomarkers would permit to stratify patients for immunomodulatory treatments should be addressed in future studies.
- The role of host genomics, microbiota as well as checkpoint inhibitor expressions will not be assessed in this study.

INTRODUCTION

Sepsis is a major health problem and the main aetiology for intensive care unit (ICU) admissions. Its incidence is increasing over the years due to several factors, including a better awareness and an ageing population. Hospital admissions for sepsis have thus overtaken those for stroke and myocardial infarction. Despite advances on its management, mortality of sepsis has remained stable over the last 20 years, reaching 30%–40% in
case of septic shock, the most severe form, and it is the leading cause of death in ICU.

Sepsis is a severe infection, defined as a ‘life-threatening organ dysfunction caused by a dysregulated host response to infection’.7 Besides circulatory and metabolic abnormalities, the multifaceted host response to the invading pathogen is amplified by comorbid conditions. It is now acknowledged that the pro-inflammatory response, which can lead to organ failure, comes with a compensatory anti-inflammatory response. Recovery occurs when inflammation resolves quickly. However, in numerous patients, the anti-inflammatory response lingers on and leads to an immunosuppression state, associated with secondary infections, and increased morbidity and mortality. This sepsis-induced immunosuppression could explain the failure of several previous clinical trials and support new innovative trials testing immune adjuvant drugs in septic shock.9

Therefore, several studies and case reports now support the rational of boosting the immune system, in order to avoid the occurrence of healthcare-associated infection and therefore reduce the associated morbidity.10 11 However, to avoid reproducing the errors from the past, such innovative treatments should be administered only to those individuals identified as immunosuppressed.11

Some studies have already demonstrated that the concept of biomarker-guided therapeutic stratification can lead to clinical improvements.12

A marked immunosuppression has been partially described in other patients admitted to the ICU for severe trauma/burns and other major surgeries.13–16 In these ‘sterile’ injuries, signs of injury-induced immune alterations have also been associated with increased susceptibility to secondary infections and mortality.

Given the complexity and heterogeneity of ICU patients, it is unlikely that any single biomarker will be sufficient to describe and diagnose injury-induced immunosuppression. On the contrary, a panel of validated biomarkers may bring enough information to accomplish such complex endeavour.

Rationale of the study

From a clinical perspective, sepsis-induced immunosuppression may be best identified by immune functional assays (such as cytokine release or lymphocyte proliferations after ex vivo stimulation) or by cell count parameters (such as number of lymphocytes or level of expression of mHLA-DR) but both approaches present drawbacks. Indeed, such functional assays are not suitable to stratify patients in a prospective interventional clinical trial due to (1) the long time to results (up to 5 days for lymphocytes proliferation) and (2) poor reproducibility due to standardisation issues and cumbersome technique. Due to such complexity, these reference tests are rarely performed in clinical studies evaluating biomarkers associated with deleterious outcomes in ICU.

On the other hand, HLA-DR expression on monocytes is currently the best biomarker available for such a routine use,17 and it is being employed for patient stratification in a large multicentre interventional trial assessing the administration of GM-CSF in patients with septic shock.18 However, its measurement requires flow cytometry analysis within 4 hours of blood sampling which may not be available in all centres, making interlaboratory standardisation challenging.

As a consequence of the previously discussed challenges, numerous biomarkers proposed to monitor injury-induced immune alterations have yet to be compared with these reference assays.

Hypothesis

Although several studies have shown an association between markers related to the immune system (eg, HLA-DR) and the occurrence of healthcare-associated infections in septic patients,14 15 19 we still do not have a clear and operational definition of the immune deficiency that occurs in severely injured ICU patients. Precise description of injury-induced immunosuppression incidence and its characteristics are lacking. In the REALISM (REAnimation Low Immune Status Markers) project, we propose to broadly assess immune parameters over time and to correlate these findings with clinical epidemiological data and outcomes in order to identify and define immunosuppression in ICU patients in terms of both magnitude and time duration.

To this aim, we have established two standardised functional immune assays (whole-blood TNF-α release after ex vivo stimulation with LPS (lipopolysaccharides)20 and lymphocyte proliferation in response to ex vivo stimulation with PHA (phytohaemagglutinin).21

We propose to define the status of immunosuppression on the basis of an abnormal result (values outside the reference intervals) obtained in at least one of the two ‘reference’ tests.

The REALISM project aims to provide a validated operational definition of injury-induced immunosuppression predicting clinically relevant outcomes. This will facilitate development of new tools and biomarkers with the goal of introducing diagnosis of immune suppression into routine clinical practice and will allow patient stratification for the evaluation of new individual immunotherapies.

It may also enable the identification of new targets and the development of new innovative therapeutics to treat ICU patients and prevent opportunistic infections in the future.
Primary aim
The primary objective of the study is to determine the incidence of injury-induced immunosuppression in ICU patients, during the first 2 months after injury.

Secondary aims
The secondary objectives of the study are as follows:
► To describe the occurrence of immunosuppression, its depth and impact on innate and adaptive immune responses and its evolution during the first 2 months after injury.
► To assess the strength of the proposed definition, in particular, by evaluating its association with secondary infections and mortality.
► To assess the accuracy of new biomarkers and immune functional assays to diagnose immunosuppression.

These new biomarkers / immune functional assays could therefore replace assays such as the T cell proliferation assay, the current protocol of which is not suited to the routine management of ICU patients. We therefore expect to provide data to validate simpler diagnostic tools to determine and follow the immune status in hospitalised patients.

Box 1  Inclusion and exclusion criteria for patients

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>Male or female aged over 18 years</td>
<td>Patient with severe neutropenia (neutrophil count &lt;0.5 G/L)</td>
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<tr>
<td>Patient hospitalised for:</td>
<td>Patients receiving immunosuppressive therapy</td>
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<td>Septic shock, defined by:</td>
<td>Use of therapeutic antibodies</td>
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<tr>
<td>Infection site suspected, and microbiological analysis sampling carried out</td>
<td>Onco-haematological disease (eg, lymphoma, leukaemia…) under treatment or treated within 5 years before inclusion</td>
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<tr>
<td>Vasopressor therapy needed to elevate mean arterial pressure ≥65 mm Hg and lactate &gt;2 mmol/L (18 mg/dL) despite adequate fluid resuscitation</td>
<td>End of chemotherapy within the 6 months prior to inclusion date</td>
</tr>
<tr>
<td>Norepinephrine &gt;0.20 µg/kg/min for at least 2 hours</td>
<td>Patient with innate or acquired immune deficiency (eg, severe combined immunodeficiency, HIV or AIDS, any stage)</td>
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<tr>
<td>Norepinephrine started within 48 hours after intensive care unit (ICU) admission</td>
<td>Patients with a ‘do not resuscitate order’ or a ‘withdraw of care’ decision, at time of inclusion</td>
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<tr>
<td>Serious trauma, defined by:</td>
<td>Patient whose anticipated duration of hospitalisation in the ICU is estimated at less than 48 hours</td>
</tr>
<tr>
<td>Patient admitted directly to the recruiting ICU</td>
<td>Participation in any interventional study</td>
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<tr>
<td>ISS, Baker et al, 1974 &gt;15</td>
<td>Extra-corporeal circulation in the month preceding inclusion in the case of cardiac surgery</td>
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<tr>
<td>Severe burns, defined by:</td>
<td>Pregnant or breastfeeding women</td>
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<tr>
<td>Total burned surface area &gt;30%</td>
<td>Patient with no social security insurance, with restricted liberty or under legal protection</td>
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METHODS AND ANALYSIS
REALISM is a prospective longitudinal, single-centre observational study, conducted in the anaesthesiology and intensive care department at the Edouard Herriot Hospital (University Hospital, Lyon, France; capacity of approximately 1000 beds).

Study population
REALISM will include healthy volunteers (n=150) and patients at risk of injury-induced immunosuppression: (1) septic shock patients (n=160), (2) severe trauma patients (n=180), (3) severe burns patients (n=30) and (4) patients admitted to the ICU after major surgery (n=180).

Septic shock inclusion criteria follow the current definition and require a state of shock defined by vasopressors administration and plasma lactate level above 2 mmol/L (18 mg/dL). An infection must be suspected, and microbiological sampling should have been performed, along with the administration of antimicrobials. Only primary septic shock will be considered (vasopressors should have been started within the first 48 hours after ICU admission).

Patients with severe trauma, defined by an ISS (injury severity score, Baker et al, 1974) >15 will be included in the study. As we hypothesised that the depth of
immunosuppression might be related to severity, we will limit the group of patients between ISS15–17 values to 90 patients to ensure that, at least, 50% of the cohort includes patients with an ISS >25. Severe burn patients will be selected for inclusion based on a total burn surface area over 30%. Surgical patients will be screened according to the planned surgical procedure. This study will include patients undergoing: (1) eso-gastrectomy, (2) Brick- er’s bladder resection (total bladder resection with reconstruction from small bowel), (3) cephalic

pancreaticoduodenectomy (Whipple’s procedure) and (4) abdominal aortic aneurysm surgery by laparotomy.

Exclusion criteria are mainly related to factors that might impact the immune status and bias the results such as the following: severe neutropenia (neutrophil count <0.5 x 10^9/L), administration of immunosuppressive therapy, corticosteroids (IV or oral administration), use of therapeutic antibodies (such as anti-TNF-α), onco-haematological disease (eg, lymphoma, leukaemia) under treatment or treated within 5 years before inclusion and end of chemotherapy within the 6 months prior to inclusion date. Patients with congenital/hereditary or acquired immune deficiency (eg, severe combined immunodeficiency, HIV or AIDS, at any stage) and patients that have received extracorporeal circulation in the month preceding inclusion will be excluded as well.

Considering the possible influence of gender bias on measured parameters, we will recruit healthy donors from both genders, following the age and gender distribution of the French population.

Complete lists of the inclusion and exclusion criteria for patients and healthy volunteers are presented in box 1 and box 2, respectively.

**Sampling schedule**

Samples and clinical data will be collected 3–4 times within the first week (early time points) with the aim to evaluate the modulation of the immune status early after injury. Samples will be collected at day 1 (the morning following injury), at day 2 (for the severe trauma group) and at day 3/4 and day 5/7 (table 1). Samples will also be collected before surgery, at day 0, as surgical patients are the only group for which sampling can be performed before injury. Additional samples will be collected during late time points to evaluate the recovery of the immune status, at day 14 (between day 13 and 18), day 28 (between day 26 and 36) and day 60 (between day 52 and 68), depending on patient availability and technical constraints (figure 1). Total volume of sampling will be 30 mL at each time point.

**Definition of immunosuppression**

The REALISM project will monitor the immune function of the patients and healthy volunteers using two standardised immune functional tests: one reference test to evaluate the innate immune response (whole-blood production of TNF-α in response to ex vivo stimulation by LPS) and a second reference test for the adaptive immune response (the lymphocyte proliferation in response to ex vivo T cell stimulation with PHA).

Immunosuppression will be defined in comparison to the values as obtained in a group of healthy volunteers for the two reference tests using the following methodology. First, reference intervals will be derived from the independent set of healthy volunteers. Second, immunosuppression will be defined in a patient when an abnormal result (value outside the reference intervals)
is obtained in at least one of the two ‘reference’ tests over at least two consecutive time points

**Definition of secondary infection**

During the ICU stay, patients will be screened daily for exposure to invasive devices (intubation, indwelling urinary catheter and central venous line) and occurrence of secondary infection. Information referent to infections will be collected, reviewed and validated by a dedicated adjudication committee, composed of three physicians not involved in the recruitment of the patients with confirmation of secondary infection made according to the definitions used by the European Centre for Disease Prevention and Control and the Infectious Diseases Society of America.

**Immune functional assays**

**Innate immune response: TNF-α release after LPS whole-blood stimulation**

Innate immune response will be evaluated by measuring the production of TNF-α in response to *ex vivo* stimulation of whole blood by LPS. The stimulation will be performed through the use of standardised TruCulture tubes from MYRIAD RBM (MYRIAD RBM, Austin, USA) (the concentration, quality and activity of the LPS is guaranteed by the manufacturer MYRIAD RBM). The tubes contain the medium alone (Null) or the medium with LPS 100 ng/mL (LPS from *Escherichia coli* O55:B5) (LPS-R; Null-R; MYRIAD RBM). The blood samples will be collected on heparin and transported to the laboratory where 1 mL of heparinized blood will be transferred to each TruCulture tube and incubated for 24 hours at 37°C. Following incubation, the supernatant will be collected using a separation valve (according to manufacturer instructions) and stored at −80°C until batch quantification of TNF-α by ELISA (BE55001; BL International-Tecan, Männedorf, Switzerland).

**Adaptive immune response: T lymphocyte proliferation after *ex vivo* peripheral blood mononuclear cells mitogenic stimulation**

Adaptive immune response will be assessed by measuring T lymphocyte proliferation in response to *ex vivo* stimulation with a mitogen. Briefly, peripheral blood mononuclear cells isolated by Ficoll density gradient centrifugation (U-04; Eurobio, Les Ulis, France) will be stimulated with PHA at 4 µg/mL (HA16; Remel, Lenexa, USA), at 37°C for 72 hours. Following incubation, the cells will be harvested and cell’s proliferation will be determined by the incorporation of EdU (5-ethynyl-2'-deoxyuridine, 10 µM for 2 hours) in T cells using the commercial kit Click-iT EdU AF488 flow kit (C10420; Life Technologies, Carlsbad, California, USA). Cell proliferation is measured as the percentage of EdU-positive T cells (gated as CD3+ using a CD3-APC staining) using flow cytometry.

**Cellular immunophenotyping**

Complete blood cell count report from the haematology laboratory will be collected on each time point, this information will be compared with our cell counts results by flow cytometry. Beside phenotypic immune cells, characterisation and cell counting will be completed by flow cytometry and we will count the number of B lymphocytes (CD45+, CD3−, CD19+), T lymphocytes, CD4+ (CD45+, CD3+, CD8−, CD4+), CD8+ (CD45+, CD3+, CD8+, CD4−), NK cells (CD45+, CD3−, CD56+), regulatory T lymphocytes (gated on T CD4+, CD25high, CD127low) and mature (CD10High, CD16High, CD14−, CCR7−) and immature mature (CD10dim, CD16dim, CD14−, CRTH2−) polymorphonuclear cells, as previously published.

In addition, the number of HLA-DR molecules per monocyte will be determined using the BDQuantibrite standardised method (HLA-DR:340827; Quantibrite:340495; Becton Dickenson, New Jersey, USA). It is well known that the flow cytometry is highly sensitive to variation between laboratories and instruments; therefore, a validation with the routine hospital immunology laboratory was performed to guarantee that all the protocols are reproducible and standardised. All procedures generated results with less than 20% of variation when compared with reference protocols.

**Biobanking**

This study will provide the opportunity to establish four different types of biobanks to preserve the material collected, enabling exploration of innovative biomarkers.

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**Figure 1** Schematic design of the REALISM project illustrating the type of patients included in the study, the various time points and major planned analysis. REALISM, REAnimation Low Immune Status Markers.
(1) TruCulture plasma biobank from whole blood stimulated with LPS, SEB (Staphylococcus aureus enterotoxin B) or not stimulated, to study cytokines release; (2) EDTA plasma biobank to study viral reactivation markers and soluble host biomarkers; (3) heparin plasma biobank for metabolomics/proteomics soluble host biomarkers studies and (4) RNA biobank to study new transcriptomic host biomarkers (RNA will be extracted from whole blood collected in PAXgene tubes).

**Innovative immune functional assays and exploration of new biomarkers**

Regarding the immune functional tests, other stimulants (eg, SEB) and read-outs (eg, interleukin 2, interferon gamma) will be tested using the TruCulture tubes. The cytokine production levels in the supernatants of the functional assays will be quantified using commercial IVD or RUO assays. Finally, a metabolomics and proteomics study will be performed using frozen (heparin) plasma. Biomarkers potentially associated to immune deficiency will be identified by liquid chromatography–mass spectrometry on high-resolution mass spectrometry and $^1$H nuclear magnetic resonance, after polar and non-polar samples extraction.

**Sample size and data analysis plan**

**Population sizing**

The number of healthy volunteers required to determine the reference intervals for the two immune reference tests was defined according to the methodology recommended by the Clinical and Laboratory Standards Institute C28-A3 guidelines. The minimal number of subjects recommended being 120, after exclusion of aberrant values (CI of 90%), we decided to include 150 healthy volunteers to take into account exclusions related to technical reasons, aberrant values or consent withdrawal.

For this reference population, the age range of healthy volunteers group has been carefully calculated to include the expected age range and gender distribution from ICU patients in France (table 1).

The main objective being descriptive, the computation of the sample size was based on secondary objectives, especially for (1) the analysis of the occurrence of immunosuppression, its depth and impact on innate and adaptive immune responses (Cohen’s d is 0.55) and (2) the correlation between new biomarkers and immune functional assays to diagnose immunosuppression ($r>0.4$). A Student’s t-test was used to approximate the number of patients needed and a minimum of 150 patients per group was required for a standardised Cohen’s d effect=0.55, if we get the recommended number of healthy volunteers of 120. It was therefore decided to include 160 septic shock patients, 180 severe trauma patients and 180 patients with a major surgery, to overcome secondary exclusions for technical causes or consent withdrawal. The severe burn patients group is an ancillary group that was arbitrary fixed at 30 subjects in order to collect data with the intent to inform a dedicated study on this population in the future.

**Statistical analysis**

First, the percentage of patients meeting the definition of injury-induced immunosuppression will be computed in each patients group to answer the main objective. Second, the occurrence of immunosuppression will be further described. The proportion of patients with at least one abnormal test will be computed for both immune reference tests and each patients group. The correlation between the two reference tests will be established from a Spearman correlation test. A mixed model will be constructed to describe the extent of the changes in the innate and adaptive measures over time, taking groups and time points into account. Third, a comparison of each biomarker or new functional tests with the two reference tests will be performed using a Spearman correlation test. For correlated biomarkers or functional tests, the performance for prediction of secondary infection will be estimated from a receiver operating characteristic curve. A Fine & Grey predictive model will be constructed for the biomarkers harbouring the best areas under curve, taking into account the competing risk of mortality. Finally, multiple imputations will be taken into consideration in the case of a relevant amount of missing values.

**ETHICS AND DISSEMINATION**

**Ethics approval**

The protocol, information documents and consent forms received approval by the local institutional review board (Comité de Protection des Personnes Sud-Est II, Bron, France) and the French National Security agency for drugs and health-related products (Approval code: 69HCL15_0379, 30November 2015). An amendment has been filled to extend sampling time points over the first week and add the metabolomics and proteomics study. This amendment has been approved on the 22July 2016 (protocol version 3). This study complies with the Declaration of Helsinki, principles of Good Clinical Practice and the French personal data protection act.

**Informed consent**

The free and informed consent of each patient and healthy volunteer will be obtained following a complete and faithful information, in comprehensive words, of the objectives, the proceedings and the constrains of the study, the right to refuse the enrollment or the possibility to withdraw at any time, when he/she is in capacity to understand. The patient (or next of kin) will also be informed of (1) the existence of processing system for data concerning them, (2) Their right to access and rectify these data (accessible through the physician of their choice) and (3) the possibility of the use of remaining biological material and associated data stored following the end of the study and their possible transfer to another academic or private party. This information is part of the written notice and the informed consent.

If the patient is not in capacity to understand and/or express his/her consent, the informed consent will be...
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<th>Table 2</th>
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<td>Serology (CMV, HSV1)</td>
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*Only for patients of the surgery group
†For the septic shock and burn patients: The enrollment at D2 will be accepted if D1 is not available
‡Only for patients of the trauma group
§Evaluation on day 0 for patients of the surgery group (not repeated on day 1)
¶Only if related to a new hospitalisation
**Also at ICU release and Hospital release
††If available
obtained from a next of kin. In the event that only the
informed consent of a third party has been sought at the
time of inclusion, the patients should be informed as
soon as possible of their participation in this study and
be asked to give their own consent to continue the study.

If the next of kin is not present and not available by phone,
the patient may be included in emergency situation.
The investigator will be required to record all steps for calling
the next of kin in the medical record (contact attempts with
date, time and phone number) and justify patient inclusion
in medical emergencies in accordance with French legisla-
tion. The written consent of the next of kin and the patient
should be obtained as soon as the person is available and as
soon as the patient’s clinical condition allows. The consent
form contains the possibility to refuse the storage of samples
after the end of the study.

Safety of participants
This study includes no serious foreseeable risk to the
health of the persons involved. The only potential risk
is related to blood sample collection (maximum 192 mL
collected over all time points — 2 months). However,
this aspect of nursing is part of daily practice. Blood
samples will be taken under the same conditions of
safety as currently used for common diagnostic tests.

Study management
The study is managed by BIOASTER and a dedicated team
composed from members of all the consortium partners.
The promoter of the study is the Hospices Civils de Lyon.
The principal investigator is Dr Thomas Rimmelé.

Data management

Clinical data
For each patient, an electronic case report form including
socio-demographic, clinical and para-clinical information
will be completed by clinical research assistants
(table 2): adescription of the hospital stay, the docu-
mation on the type of injury (surgery, burn, trauma
or septic shock) and the severity as defined by the ASA
classification, SOFA score and SAPSII score. In addi-
tion, we will collect routine laboratory results about the
CMV, HSV1 serology and complete blood count. More-
over, we will document if there is any specific treatments
administered to the patient, such as antibiotics, exposure
to invasive medical devices and secondary infections.
All data will be transferred to a TranSMART database
following curation for data exploration and analysis.

Duration of the study
The study is planned to run for 30 months, starting
December 2015. The expected end date for recruitment is
June 2018. Some biomarkers will be quantified by batch
analysis, at the end of the study. Primary data analysis is
expected to be completed with subsequent dissemination
of results by December 2018.

Author affiliations
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Contributors
All authors (M-LR, JT, VM, PC, LO, DG, AG, AP, JT and GM) fulfilled
ICMJE guidelines and provided substantial contributions to conception, design and
acquisition of data; drafted and revised critically the manuscript; and approved the
final version of the manuscript.

Competing interests
AP, JT and VM are employees of bioMérieux SA, an in vitro
diagnostic company. PC, LO and DG are employees of SANOFI-AVENTIS R&D, SANOFI-
Pasteur SA and GlaxoSmithKline, three pharmaceutical companies.

Ethics approval
Comité de Protection des Personnes Lyon Sud-Est 2.

Provenance and peer review
Not commissioned; externally peer reviewed.

Data sharing statement
Results will be disseminated through presentations at
scientific meetings and publications in peer-reviewed journals. New markers and
immune functional tests will be evaluated for the diagnostic immune deficiency and
may be patentable.

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The REAnimation Low Immune Status Markers (REALISM) project: a protocol for broad characterisation and follow-up of injury-induced immunosuppression in intensive care unit (ICU) critically ill patients

Mary-Luz Rol, Fabienne Venet, Thomas Rimmele, Virginie Moucadel, Pierre Cortez, Laurence Quemeneur, David Gardiner, Andrew Griffiths, Alexandre Pachot, Julien Textoris and Guillaume Monneret

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