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### Immune dysfunction in sepsis



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An overview of the recent advances in the diagnosis and treatment of immune system dysfunction in sepsis.

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al. 2016). Sepsis-induced immune system dysfunction is an important sequelae of sepsis. The persistence of immune system dysfunction in the later stages of sepsis increases patients' susceptibility to secondary infections and, consequently, leads to an increased mortality (Boomer et al. 2011; Torgersen et al. 2009; Zorio et al. 2017; Cavassani et al. 2010). In recent years, there have been major advances in the research of sepsis-induced immune dysfunction. The purpose of this article is to review how these recent advances could be translated into future, better ways of diagnosing and treating immune system dysfunction in sepsis patients.

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## Diagnosis of immune system dysfunction

A myriad of immune deficits has been identified in sepsis patients. Here we describe the major immune deficits that can be measured by either well-established biomarkers or readily available laboratory methods. While the evidence base for these measurements remains incomplete, the methods listed below represent some of the most promising advances made in recent years.

### 1. HLA-DR

**What has the research shown?**

Expression of monocytes HLA-DR has been shown to be a useful biomarker of immune dysfunction (Monneret et al. 2008). HLA-DR is a major histocompatibility complex (MHC) class II cell surface receptor found on antigen presenting cells. Its role is to present peptide antigen to elicit T helper response. Low mHLA-DR levels have been associated with a higher risk of developing nosocomial infections and a higher mortality in sepsis patients ( Landelle et al. 2010; Monneret et al. 2006; Caille et al. 2004).

**What is the diagnostic method?**

Monocytes HLA-DR can be measured by flow cytometry; however, this remains problematic due to high inter-laboratory variance (25%) (Docke et al. 2005).

### 2. PD-L1 and PD-L2

#### **What has the research shown?**

Programmed death-1 (PD-1) and its associated pathway negatively control immune responses. Up-regulation of the PD-1 gene (in T cells) and its ligands, PD-L1 and PD-L2 (antigen presenting cells) may impair adaptive immune response. Overexpression of these molecules has been shown to correlate with increased secondary nosocomial infections and adverse outcomes in septic patients (Zhang et al. 2011; Guignant et al. 2011).

#### **What is the diagnostic method?**

Specific monoclonal antibody binding to PD-L1 and PD-L2 and flow cytometry analysis can be used.

### **3. Cytokines**

#### **What has the research shown?**

Measuring either pro- or anti-inflammatory cytokines may aid the diagnosis of immune dysfunction. For example, increased anti-inflammatory cytokine IL-10 production was associated with reduced expression of HLA-DR and PD-L1 on monocytes and PD-1 on T-cells ( Guignant et al. 2011).

#### **What is the diagnostic method?**

Commercially available assays for detection and analysis of cytokines are the enzyme-linked immunosorbent assays (ELISA), the enzyme-linked immune spot (ELISPOT) assay, and the polymerase chain reaction (PCR) method to determine gene expression for cytokine production. For better evaluation of the complex inflammatory response, researchers can use multiplex-based immunoassays, such as the bead-based immunoassay read by flow cytometer or micropatterned antibody cytokine array (Stenken and Poschenrieder 2015). As another alternative, intracellular cytokine staining (by flow cytometry analysis) has also been used in some studies (Monneret and Vennet 2016).

### **4. Immunoglobulin**

#### **What has the research shown?**

Hypo-gammaglobulinaemia has been a frequent finding in patients with sepsis. Low levels of immunoglobulin isotypes (IgG, IgA, IgM) has strongly correlated with prognosis (Tamayo et al. 2012; Prucha et al. 2013).

#### **What is the diagnostic method?**

Quantitative serum immunoglobulin tests are used to detect abnormal levels of the three major classes (IgG, IgA and IgM). Among the tests available, nephelometry and turbidimetry are the most widely used methods because of their speed, ease of use and precision (Loh et al. 2013).

## 5. Lymphocytes

### What has the research shown?

Lymphocytes from septic patients have been found to be anergic, i.e. lacking the late-phase hyper-reactivity in the intradermal tests. This “anergic” state was associated with an increased vulnerability to secondary infections and mortality (Christou et al. 1995). Septic T cells also demonstrated a low proliferation rate in vitro (Lederer et al. 1999). However, as proliferation tests require a long incubation time, they are not routinely performed (Monneret et al. 2011).

### What is the diagnostic method?

Lymphocyte counts may reflect immune cell apoptosis in sepsis (Cheadle et al. 1993; Rajan and Sleight 1997). Lymphopaenia following the diagnosis of sepsis or persistent lymphopaenia may therefore serve as a biomarker for immune dysfunction (Drewry et al. 2014; Parnell et al. 2013).

## 6. Signature gene-expression markers

### What has the research shown?

HLA-DR mRNA gene expression may be a valuable diagnostic tool to overcome the methodological difficulties with flow cytometry described earlier (Le Tulzo et al. 2004; Monneret et al. 2004; Cajander et al. 2015). In addition, anti-apoptotic Bcl-2 gene expression in patients with sepsis has been shown to be associated with a reduced T-cell count and may also be used as a marker of immune dysfunction (Billbault et al. 2004). Other potential gene-expression biomarkers, such as T-bet, GATA-binding protein 3 (GATA3), CTLA-4, PD-1, PD-L1, PD-L2, CD47, CD3, CD28 and LAG-3, were also associated with changes in the CD4+ T cell response in sepsis patients (Zhang et al. 2011; Guignant et al. 2011; Roger et al. 2009; Hotchkiss and Karl 2003; Venet et al. 2005).

### What is the diagnostic method?

These markers can be measured by real-time PCR, which is available in most hospital laboratories.

## 7. Host response “endotypes”

### What has the research shown?

Recent transcriptomic analysis of the peripheral blood of sepsis patients revealed two distinct sepsis response signature (SRS) groups, termed “SRS1” and “SRS2” by the authors of the study (Davenport et al. 2016). The presence of SRS1 (detected in 108 [41%] patients) identified individuals with an immunosuppressed phenotype that included features of endotoxin tolerance, T-cell exhaustion, and downregulation of HLA-DR. The SRS1 group had a higher 14-day mortality with a hazard ratio of 2.4 (95%CI 1.3–4.5). There were similar findings from a more recent transcriptomic study, in which the study authors used a 140-genes signature to stratify sepsis patients into four endotypes. One endotype was associated with a higher 28-day mortality in septic patients,

with a hazard ratio of 1.86 (95% CI 1.21-2.86) (Sciocluna et al. 2017). Both these studies indicated that sepsis “endotypes” (distinctive gene clusters of immune pathways) correlate with the degree of immune dysfunction and adverse outcomes in sepsis patients.

### **What is the diagnostic method?**

Whole genome transcriptomic analysis (using peripheral blood samples) can be used to measure sepsis endotypes in patients.

## **Treatment of immune system dysfunction**

Ideally, the immune modulation therapy needs to be sufficiently broad to correct the widespread immune defects in sepsis, but at the same time must be titratable to prevent untoward immune system activation. Personalised immune profiling, as outlined in the previous section, will allow clinicians to titrate the immune therapy and correct any inadvertent alterations in immune defence.

Next, we will highlight several previously studied therapies as well as therapies that are currently in the development stage;

### **1. Established immune modulating agents**

One of the oldest immune modulating agents is the cytokine IFN $\gamma$ . In a seminal study, Docke et al. (1997) reported that IFN $\gamma$  administered to septic patients with low monocyte HLA-DR expression on monocytes restored HLA-DR expression and resulted in clearance of sepsis in eight of nine patients. Since then, a relatively small number of septic patients have been treated with IFN $\gamma$ , including those with persistent staphylococcal and invasive fungal infections (Delsing et al. 2014; Nalos et al. 2012). However, there is no randomised controlled trial data available for IFN $\gamma$ .

Another cytokine that has been studied is a haematopoietic growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF therapy reversed defective TNF $\gamma$  response, T-cell anergy and prevented nosocomial or recurrent infections in children. In adults, GM-CSF treatment restored HLA-DR expression on monocytes, and reduced secondary infections and duration of hospital stay (Rosenbloom et al. 2005; Orozco et al. 2006). Meisel and colleagues tested the efficacy of GM-CSF in patients with decreased monocyte HLA-DR expression (Meisel et al. 2009). Additionally, a clinical trial (GM-CSF to Decrease ICU Acquired Infections - GRID trial) evaluating this therapeutic approach in septic shock patients is soon to be completed ([clinicaltrials.gov/ct2/show/NCT02361528](https://clinicaltrials.gov/ct2/show/NCT02361528)).

IL-7 is another potential therapeutic agent, with recombinant human cytokine IL-7 (rhIL-7) being investigated for sepsis immunotherapy. Treatment with rhIL-7 demonstrated improved T cell proliferation, enhanced lymphocyte metabolism and IFN $\gamma$  production ex vivo in septic patients (Venet et al. 2017; 2012). Based on these promising results, a phase 2 multicentre randomised controlled trial (IRIS trial) assessing rhIL-7 in patients with septic shock was designed (A Study of IL-7 to Restore Absolute Lymphocyte Counts in Sepsis Patients (IRIS-7-B) - [clinicaltrials.gov/ct2/show/NCT02640807](https://clinicaltrials.gov/ct2/show/NCT02640807)); its primary aim is to ascertain the safety and ability of rhIL-7 to increase the absolute lymphocyte count in immunosuppressed septic patients.

### **2. New immune modulating agents**

CTLA-4, PD-1, PD-L1 and LAG-3 are potent immune cell inhibitors that are highly upregulated in septic patients. Immune checkpoint inhibitors are antibodies, originally used in cancer treatment, that target these key signalling pathways. These include anti-PD-1 antibodies (nivolumab and pembrolizumab) and anti-PD-L1 antibodies (atezolizumab, avelumab and durvalumab). These agents have been shown to reverse the exhausted cytotoxic T cells in cancer patients, thereby restoring T cell function; therefore, this approach may be a promising

therapeutic strategy in sepsis (Chang et al. 2014; Zhang et al. 2010). Ex vivo studies using cells from septic patients' cells have shown that a PD1/PD-L1 pathway blockade decreased sepsis-induced immune dysfunctions (Chang et al. 2014; Zhang et al. 2010). However, immune checkpoint inhibitors could cause autoimmune adverse events, which are driven by the same immunologic mechanisms responsible for their therapeutic effects. Serious and life-threatening autoimmune events are reported in the literature with treatment-related deaths of up to 2% of cancer patients (Puzanov et al. 2017).

Thymosin alpha-1 (Ta1), and Flt3L protein are molecules that can induce appropriate dendritic maturation and T cell activation (Flohé et al. 2006). In septic animals, Flt3L increased dendritic cell numbers and IL-12 production in these cells, thus enhancing CD8 T cells responses (Strother et al. 2016). A recent report demonstrated the efficacy and safety of recombinant human Flt3L (Anandasabapathy et al. 2015). Ta1 also induces the maturation of dendritic cells and T cells maturation. In a recent meta-analysis, Li et al. (2015) evaluated the results of twelve controlled trials using Ta1 in sepsis and found a trend towards lowering all-cause mortality. Clearly, further studies are needed to explore the therapeutic potential of these molecules.

## Summary

The complexity of sepsis-induced immune dysfunction is now being unravelled by rapid advances in the “omics” sciences (e.g. transcriptomics). In the near future, novel biomarkers will be used to measure specific immune deficits in sepsis patients. Furthermore, promising immune therapies (e.g. checkpoint inhibitors) are also currently being investigated in pre-clinical studies. Validation of these new diagnostics/therapeutics in a clinical trial setting will be an important next step.

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## Conflict of interest

Velma Herwanto, Marek Nalos, Anthony S. McLean and Benjamin Tang declare that they have no conflict of interest.

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