Which host response assays are ready to get implemented in clinical practice?

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What are host response assays?

To monitor host immune status;

• Adaptive immune responses;

• Innate immune responses;

• Systemic;

• Local or tissue level;
The key role of the immune system in maintaining homeostasis

The immune system is a dynamic system that maintains the integrity of the body;

Yet, unbalanced responses may lead to many diseases:
   Poor or inefficient responses:
      Opportunistic infections;
      Microbial reactivations;
      Enhanced susceptibility to infections;

Exacerbated responses:
   Autoimmune diseases;
   Allergies;
   Chronic inflammatory diseases
What are host response assays?

In Infectious Diseases clinical practice:

When there are limitations in the direct diagnosis of a given infectious disease as for:
• Agents commonly associated with the host;
• Agents that may establish a latent infection;
• Instances in which the clinical outcome spans a large spectrum of conditions;

Host response assays may aid in:
• Diagnosis of infections;
• Prediction of secondary infection;
• Ascertain or predict protection;

The ability to closely monitor the immune status is thus a critical unmet medical need, which may help stratify patients for personalized care.
Immune Functional Assays
aim to assess host immune function by measuring one or more immunological parameters

• Antigen-specific immune responses (humoral or cellular);
• Characterization of distinct immune cell (sub)populations (including in vivo detection of immune cells infused for therapeutic functions, T Reg);
• T-cell and B-cell regulatory networks
• Innate immune responses
• Markers of T-cell turnover and homing to organs and/or lymphoid tissue
• Cytokine and signaling networks
• Gene and protein expression and regulation
• Mucosal inflammatory and innate immune responses
What technologies shall ideally be used to enter clinical practice

Ideally, the technology shall be:

- Performed on easy to acquire biological samples and in small volumes;
- Relatively simple to perform;
- Easy to standardize;
- Scalable;

- Microfluidic platforms
- Novel cell-based assays
- Multiplexed phenotypic imaging for various cell function studies
- Multiplexed mass cytometry (CyToF) profiling
- Multiplexed fluorescence/mass microscopy techniques
- Highly multiplexed flow cytometry approaches
- Nanotechnology-based biosensing platforms
- New microarray technologies
- Next generation sequencing technologies

Diagnostic assays  Mechanistic assays
Tuberculin Skin Test — Developed between 1890 - 1908
The first Immune Functional Assay for the diagnosis of *M. tuberculosis* infection
Interferon-Gamma Release Assays (IGRAs)
Mtb antigens

T cell immune responses

T cell immune responses
The challenges associated with host response assays

- Many conditions can affect the host immune response in healthy subjects and patients;
- Blood can provide an average of quite different conditions occurring at tissue level;


Dyrhol-Riise AM et al (2010) BMC Infectious Diseases

Heterogeneity of TB disease

TB spectrum
Lessons learned:

• Improved diagnosis;
• Helped to understand the dynamic host-pathogen interactions during *Mtb* infection and TB disease;
• TB heterogeneity;

Unmet need:

• Dissect further the Mtb infection dynamic;
• Identify immunologic correlates of risk;
• Identify immunological correlates of protection
Biomarkers:

- Antigens;
- Cytokines;
- Cell subpopulations;
- Metabolites;
• Children with QFT conversion at IFN\(_\gamma\) values >0.35 and ≤4 IU/ml did not have significantly increased risk of disease;

• Conversion at QFT with IFN\(_\gamma\) values > 4 IU/ml were associated with substantially increased disease incidence;
IFNγ response to QFT antigens and HBHA response in LTBI children.

Sali M., Buonsenso D. et al. (2018) J. Infection
Following TB-specific therapy, most of the non-HBHA-responding children, gained an HBHA- positive response.

HBHA-based IGRAs TO MONITOR TB THERAPY

Sali M., Buonsenso D. et al. (2018) J. Infection
Whole-blood transcriptional gene signatures in TB

TB signature was dominated by a neutrophil-driven interferon (IFN)-inducible gene profile, consisting of both IFN-γ and type I IFN-αβ signalling.

Diagnostic signatures

1. **Donor enrollment**
   - Diseases closely related for differential diagnosis (e.g., sarcoidosis)
   - Representative cohort of target population
   - Large cohorts

2. **Sample collection**
   - Readily accessible with high RNA content (e.g., blood)
   - Unfractionned samples

3. **Data generation**
   - Whole-transcriptome
   - Validation of selected genes by RT-qPCR
   - *Ex vivo* analysis preferred

4. **Data analysis**
   - High stringency
   - Top classifier genes

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Burel JG et al (2019) Frontiers in Immunology


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The whole blood tuberculosis risk signature prospectively identified people at risk of developing active tuberculosis, opening the possibility for targeted intervention to prevent the disease.
Systemic inflammation is a whole body reaction having an infection-positive (i.e., sepsis) or infection-negative origin.

It is important to distinguish between these two etiologies early and accurately because this has significant therapeutic implications for critically ill patients.

Ideally, a molecular classifier based on peripheral blood RNAs shall:
- determine which patients with systemic inflammation had sepsis;
- be robust across independent patient cohorts;
- be insensitive to disease severity;
- Provide diagnostic utility.
SeptiCyte Lab, a peripheral blood-based molecular assay, has been shown to be rapid, robust, and accurate for differentiating cases (ICU patients retrospectively diagnosed with sepsis) from controls (ICU patients retrospectively diagnosed with infection-negative systemic inflammation).

SeptiCyte Lab, a Host Gene Expression Signature Discriminates Clinical Severe Sepsis Syndrome and Infection-Negative Systemic Inflammation Among Critically Ill Children

Discrimination of clinical severe sepsis syndrome (CSSS) from postcardiopulmonary bypass (PCPB) based on next-generation sequencing data.

Discrimination clinical severe sepsis syndrome (CSSS) from postcardiopulmonary bypass (PCPB), based on reverse transcription quantitative polymerase chain reaction (RT-qPCR) data.


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SeptiCyte Lab, a Host Gene Expression Signature Discriminates Clinical Severe Sepsis Syndrome and Infection-Negative Systemic Inflammation Among Critically Ill Children

Stratification SeptiCyte Lab test score, based on culture results.

PCPB: postcardiopulmonary bypass; CSSS: clinical severe sepsis syndrome


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Evaluation of the diagnostic performance of the molecular host response assay (SeptiCyte LAB) designed to distinguish between sepsis and noninfectious systemic inflammation in critically ill adults.

SeptiCyte LAB appears to be a promising diagnostic tool to complement physician assessment of infection likelihood in critically ill adult patients with systemic inflammation;

SeptiCyte LAB is the first host response gene expression assay cleared by the U.S. Food and Drug Administration as an aid for diagnosis of sepsis.

Blue = models containing SeptiScore;
Green = models containing procalcitonin but not SeptiScore;
Red = models without SeptiScore or procalcitonin.
Blood Signature to Discriminates Systemic Inflammation Due to Viral Infection Versus Other Etiologies

Time-course of pan-viral signature score for human volunteers vaccinated with live attenuated yellow fever vaccine.

Pan-viral signature score for children with acute RSV infection and following recovery.

Pan-viral signature score in adult patients presenting to the emergency department with fever.

Sampson DL et al (2017) Scientific Reports

Delogu G ECCMID 2019
Conclusions

• Host response assays can be very helpful in the management of many infectious diseases, particularly in complex patients;
• Host response assays may help stratify patients for personalized care;
• However, a major issue is the intrinsic variability and heterogeneity of host immune responses in healthy subjects and patients;
• Implementation in clinical practice requires extensive studies including validation in different and multiple cohorts;
• IGRAs and SeptiCyte Lab have been licensed as an “aid in the diagnosis of...”, implying the need of using other clinical and/or (micro)biological parameters to make diagnosis;