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Transcriptional profiling in infectious diseases: Ready for prime time?

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Summary Blood represents a reservoir and a migration compartment of cells of the immune system. Traditional microbiologic diagnostic tests relied on laboratory identification of the pathogen causing the infection. However, this approach is less than optimal for a variety of reasons: pathogen's slow growth, resistance to cultivation *in vitro* or insufficient proof to establish causality when a pathogen is identified. An alternative approach to the pathogen-detection strategy is based on a comprehensive analysis of the host response to the infection by analysis of blood leukocytes gene expression profiles. This strategy has been successfully applied to distinguish and classify children and adults with acute infections caused by different pathogens. Molecular distance to health (MDTH) is a genomic score that measures the global transcriptional perturbation in each individual patient compared to healthy controls. Studies indicate that MDTH is a promising biomarker to help classifying patients according to clinical severity.

The need for improving diagnosis in febrile illnesses

One of the most frequent challenges that physicians, especially paediatricians, face in the clinical setting is the difficulty in establishing an appropriate etiologic diagnosis, or even distinguishing between bacterial or viral infections, in patients presenting with an acute febrile illness. The need to promptly start appropriate antimicrobial therapy in order to control a mild infection before it can progress to a more severe form has to be balanced with the need for prudent use of antibiotics, specially in the current situation where outbreaks of emergent and re-emergent pathogens are linked to increased resistance to our current antimicrobial armamentarium. Further argument for judicious use of antimicrobial agents is the evolving information suggesting that antimicrobial therapy, most notably early in life,

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can have a major impact altering the gut microbiome that can lead to abnormal immune development. Within this context, there is a need for improved analytical tools that can advance our ability to diagnose and classify patients with infectious diseases more precisely, which in turn should allow for more appropriate use of antimicrobial agents.

An alternative strategy for diagnosis in infectious diseases

Our inability to identify infectious agents to establish an appropriate diagnosis remains inadequate, particularly if the organism is not present in the blood or other easily accessible site. These diagnostic obstacles can delay initiation of appropriate therapy which can result in unnecessary morbidity and even death.[1] The traditional microbiologic diagnostic tests have relied on laboratory identification of the pathogen causing the infection. Microbial pathogens are detected in clinically-relevant specimens using a variety of assays including cultures, rapid antigen detection tests, and more recently polymerase chain reaction (PCR) assays. To date, growth of the specific pathogen (bacteria, virus and fungus) remains the ultimate reference gold standard for their identification. However, many pathogens grow slowly or require complex media,[2] and a significant number of clinically-important microbial pathogens remain unrecognized as they are resistant to cultivation in the laboratory, limiting the physician's clinical decision-making.[2,3] The introduction of more sensitive molecular diagnostic assays has dramatically improved our ability to diagnose viral infections.[4] Unfortunately, this has not been the case for bacterial pathogens. Moreover, in the clinical scenario is not uncommon to encounter situations in which the sole identification of a pathogen is not sufficient to establish causality, e.g. the detection of respiratory viruses in patients with pneumonia, which in many occasions are found in patients with possible bacterial co-infections.

An alternative approach to the traditional pathogen-detection strategy is based on a comprehensive analysis of the host response to the infection caused by different microbial pathogens.[5,6] Different classes of pathogens trigger specific pattern-recognition receptors (PRRs) differentially expressed on leukocytes.[7,8] Leukocytes are components of the innate immune system (granulocytes, natural killer cells), the adaptive immune system (T and B lymphocytes), or both (monocytes and dendritic cells). Blood represents both a reservoir and a migration compartment for these immune cells that become educated and implement their function by circulating between central and peripheral lymphoid organs and migrating to and from the site of infection via blood. Therefore, blood leukocytes constitute an accessible source of clinically-relevant information, and a comprehensive molecular phenotype of these cells can be obtained using gene expression microarrays.[9] Because they provide a comprehensive assessment of the immune-related cells and pathways, genomic studies have shown to be well suited to study the host—pathogen interaction. In fact studies have shown that different classes of pathogens induce distinct gene expression profiles that can be identified by analyses of blood leukocytes (Fig. 1).[6,10–13]

Proof of concept in humans

The initial evidence supporting the hypothesis that pathogen-specific gene expression profiles can be measured in immune cells was derived from *in vitro* studies. A number of experimental studies demonstrated that different transcriptional programs could be triggered upon exposure of immune cells to various pathogens *in vitro*.[14–17] When those initial *in vitro* studies were published, there was a major interest in rapidly translating those findings to the clinical setting, but also remarkable skepticism among other investigators.

Studies tested the hypothesis that leukocytes isolated from peripheral blood of patients with acute infections carry unique transcriptional signatures, which would in turn permit pathogen discrimination and thus patient classification.[6] In those initial studies gene expression patterns in peripheral blood mononuclear cells (PBMCs) from 95 pediatric patients with acute infections caused by four common human pathogens were analyzed: a) influenza A, an RNA virus; b) *Staphylococcus aureus*, and c) *Streptococcus pneumoniae*, two Gram-positive bacteria; and d) *Escherichia coli*, a Gram-negative bacterium.

Pair-wise comparisons and class prediction analysis (K-NN algorithm)[18,19] identified 35 genes that discriminated patients with influenza A virus infection from patients with bacterial infection caused by either *E. coli* or *S. pneumoniae* with 95% accuracy.[6] Further analyses in this cohort of patients allowed us to identify 137 classifier genes, which were applied to a population of 27 pediatric patients with pneumonia and 7 healthy children to determine whether we could differentiate patients presenting with similar symptoms but according to the different etiologic pathogens (Fig. 2). Hierarchical clustering of genes and samples identified four prototypical expression profiles: (1) healthy controls (2) influenza A infection, which showed increased expression of interferon-inducible genes and was clearly different from a third profile, (3) which characterized bacterial infections caused by *S. aureus* and *S. pneumoniae*, which showed over-expression of neutrophil-associated genes. Three samples belonging to the influenza A group and 1 from the *S. aureus* group were characterized by a fourth profile, which combined elements of the previous ones, suggesting the possibility of a co-infection caused by both a viral and a bacterial pathogen.[6] These initial studies demonstrated that blood leukocytes gene expression patterns can be used to distinguish patients with acute infections caused by four of the most common pathogens leading to hospitalization in children.

Once these initial studies established the value of this strategy, the next steps are aimed at conducting larger studies in the most relevant clinical situations, where the application of this methodology has the potential to transform the standard of care. In this respect, the evaluation of young febrile infants less than 2 months of age who present to the emergency department (ED) continues to represent a major challenge for clinicians. For this reason, the Pediatric