Host transcriptomics for diagnosis of infectious diseases: one step closer to clinical application

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One of the most common and challenging situations that physicians face in their daily clinical practice is the diagnosis and management of patients with acute respiratory infections. Until recently, the most common and "practical" approach followed by clinicians was to treat the majority of these patients empirically with antibiotics. The pragmatic argument has been that antibiotics are prescribed to treat the bacteria causing the respiratory infection, even if symptoms suggest a viral infection because of the risk of a concomitant or potential superimposed bacterial infection. Arguments against this practical approach have become more evident in recent years as new studies have shed light on the true impact of this strategy. Traditionally, most concerns regarding the use of unnecessary antimicrobial agents have been related to the development of resistant bacteria [1]. This concern was mostly perceived as an epidemiological, global issue, not affecting the individual patient whose symptoms need to be addressed at that precise moment. Studies in the last decade, however, have revealed the negative effects of antimicrobial therapy on the composition of the microbiome of the individual patient and its potential consequences [2, 3]. We learned the importance of the microbiome in directing the normal physiological maturation of the immune system and how changes on its composition can increase the risk of a number of immune-mediated and infectious conditions affecting the gastrointestinal and respiratory tracts. Furthermore, studies have shown that excessive antibiotic use can be associated with poor outcomes in critically ill children [4, 5]. This evidence has dramatically shifted the perception of the impact of inappropriate antibiotic use, from a vague global concern to the individual patients we treat every day in our clinical practice. This new awareness has encouraged the establishment of new antimicrobial stewardship programmes aimed at educating clinicians about the appropriate use of antimicrobial agents [6, 7].

Management of respiratory infections is an obvious area that requires innovative approaches to help reduce the use of unnecessary antibiotics. To accomplish this goal, there is a clear need to develop and implement new diagnostic strategies that empower clinicians to target antimicrobial therapy to those patients who truly need it. In recent years, the introduction of molecular, PCR-based methods has revolutionised diagnostic microbiology. We have experienced major improvements in the ability of these

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assays to detect and quantify viral pathogens in many diseases using a variety of clinical samples [8]. In the context of respiratory infections, these assays permit detection of a large number of respiratory viruses in the upper and lower respiratory tract with great precision [9–11]. Despite these improvements in viral identification, the diagnosis of bacterial infections remains suboptimal. This is especially true in patients with lower respiratory tract infections where the detection of certain viruses in respiratory samples, especially from the upper respiratory tract, cannot exclude the presence of a concurrent bacterial infection.

The limitations of pathogen-based assays have led to the development of alternative diagnostic methods based on the assessment of the host response to infection. With this new approach, the diagnosis is established by measuring the differences in blood RNA expression profiles induced by different types of viral and bacterial pathogens [12]. Each pathogen induces a distinct RNA expression pattern that allows discrimination between type of pathogens (*e.g.* bacterial or viral) and even between specific pathogens (*e.g. Escherichia coli* or *Streptococcus pneumoniae*; influenza or respiratory syncytial virus and rhinovirus) [13–22].

A number of studies conducted in children and adults have established the proof of principle and demonstrated the potential value of this approach to discriminate between viral and bacterial infections, and among different types of bacteria and viruses. To achieve a precise discrimination between types of infections, most studies first identified a number of biomarker gene transcripts and then applied them to a different cohort of patients in a validation step. The number of biomarkers required to achieve adequate discrimination was variable (depending on the condition) but was usually more than 10–20 transcripts [13–22], except for a recent study in febrile children in which only two transcripts were sufficient to discriminate children with confirmed viral from those with confirmed bacterial infections [19].

Measuring global patterns of host response to infection represents a new paradigm for the diagnosis of infectious diseases. Before this attractive methodology can be incorporated into clinical practice, there are important issues that need to be addressed, including validation in larger and more diverse patient cohorts, and feasibility. One important aspect that will facilitate its clinical implementation would be to simplify these assays: 1) by reducing the number of biomarkers required for an optimal diagnosis and 2) by using molecular platforms that are easier to operate in the clinical laboratories with a faster turnaround time. In this issue of the *European Respiratory Journal*, Tang *et al.* [23] report a study that demonstrates significant progress in these two areas. They identified *IF27* (interferon-induced protein 27) as a single immune biomarker to discriminate patients with influenza infection from patients with bacterial infections. This is a large and carefully designed study that included a large number of subjects (n=1071) enrolled in several cohorts in three different countries. First, using microarrays, they analysed an initial cohort of patients with influenza infection and compared them with healthy controls, and found that among all differentially expressed genes, *IF27* was the biomarker with the best diagnostic performance to discriminate between these two groups. They confirmed this observation in two additional cohorts using, also microarray-based assays, and subsequently in three discovery cohorts (n=111) using a PCR-based assay. In addition and after this initial exercise, they performed a series of validation studies. In phase I validation, they analysed four previously published datasets that included both paediatric (n=230) and adult patients (n=291) with influenza infection. In three of those four cohorts, *IF27* was the most overexpressed gene, and it was the second most overexpressed in the fourth cohort. Subsequently, to identify which immune cells expressed *IF27*, they performed *in vitro* experiments and stimulated eight different immune cell subsets with influenza antigen. These experiments demonstrated that plasmacytoid dendritic cells (pDCs) expressed the highest levels of *IF27*, *via* Toll-like receptor 7. Similar experiments showed that stimulation with lipopolysaccharide did not induce significant *IF27* expression in immune cells, suggesting the potential of *IF27* to discriminate influenza from bacterial infections, and in fact, they showed that *IF27* discriminated patients with influenza pneumonitis from those with bacterial pneumonia infection (in the discovery cohorts). With these findings, the investigators performed a phase II validation in a newly enrolled prospective cohort (402 patients and 37 healthy controls) in which they tested the diagnostic value of *IF27* expression using a PCR-based platform. Using this PCR platform with the optimal cut-off value for *IF27*, they discriminated patients with influenza from those with bacterial infection with a sensitivity of 0.80, specificity of 0.90 and area under the curve (AUC) of 91%. They also examined the performance characteristics of the PCR platform in patients with influenza infection *versus* those with multiple aetiologies (bacteria, other viruses and noninfectious aetiologies), and showed a sensitivity of 0.80, specificity of 0.80 and AUC 88%. Although there were small numbers of patients with other viral infections, they examined the performance of *IF27* expression in patients with influenza *versus* other viral infections, and found a sensitivity of 0.80, specificity of 0.75 and AUC 83%.

This is a remarkable study that provides further evidence of the value of host transcriptome analysis to improve the diagnosis of patients with acute infections. The investigators identified *IF27* as a robust biomarker in patients with influenza using microarray-based assays; they analysed its expression with *in vitro* experiments in immune cells and recognised the highest expression in pDCs; and finally, they

validated its diagnostic value using a PCR-based platform that can be easily implemented in the clinical setting in a large number of patients recruited in different countries. The data presented discriminating influenza from bacterial infections are quite robust. As the authors indicated, the ability of *IF27* to discriminate between influenza and other viral infections is not yet as compelling, which is not unexpected as *IF27* is highly expressed in other viral infections [17, 24]. It would be also desirable to evaluate its ability to discriminate influenza from patients with viral–bacterial coinfections. Nevertheless, despite these limitations and the need for further studies in additional patient populations, and possibly using more than a single biomarker, this study represents an important step in advancing the application of host transcriptomics as a new diagnostic tool in the clinical setting.

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