Pathogen identification by nuclear imaging – almost there?

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Published online: 1 June 2012
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Invasive fungal disease (IFD) in immunocompromised patients is a major infective cause of morbidity and mortality, with rates of death up to 90 % [1]. A key factor is the challenge of early diagnosis of IFD. In patients with haematological malignancy following intensive chemotherapy, immunosuppression and/or allogeneic haematopoietic stem cell transplantation, it is rarely possible to obtain the diagnostic material to definitively establish the presence of an IFD. The reasons for this are multiple, and include profound pancytopenia and poor performance status, which make tissue sampling of the lung (the main site for invasive aspergillosis, IA) hazardous. Equally, laboratory biological tests for IA have shown limited efficacy in the setting of blood sampling [2, 3], whereas bronchoalveolar lavage fluid appears to be a better material to assay, but bronchoscopy is an invasive intervention and may not be possible in an ill patient. All these factors, combined with post-mortem data showing higher rates of IFD than diagnosed ante-mortem [4], have led to empirical therapy as the standard of care in many centres around the world. The definition of empirical IFD therapy used throughout the literature is the commencement of treatment based on the clinical scenario alone, with the commonest situation being persistent neutropenic fever despite the use of broad-spectrum antibiotics.

However, building on recent clinical studies [5–7], current management algorithms for IFD emphasize the

importance of attempting to make a diagnosis, rather than relying on an empirical approach alone [8, 9]. CT scanning of the chest has a central role in the management of IA, with characteristic findings (nodules with or without a halo, consolidation with cavitation or an air crescent sign) supporting the diagnosis of IFD [10]. However, as the gold standard of a histological diagnosis or culture from sterile material is rarely possible, it becomes immediately obvious that even in a diagnosis-driven strategy combining imaging and galactomannan detection (the only widely used biomarker for IA), treatment is usually given without definitive proof of IA! Furthermore, while imaging of the chest with CT can support the diagnosis of IA, there are numerous alternative infections, as well as other disease processes; and galactomannan detection itself is associated with false-negatives, false-positives and issues of reproducibility.

The paper by Petrik et al. [11] offers hope of moving the field forward by combining imaging with pathogen detection. They have exploited the dependence on iron of many microorganisms, including *Aspergillus fumigatus*. In iron-limiting environments, *A. fumigatus* produces large amounts of siderophores, which are iron-chelating peptides that scavenge Fe^{3+} and facilitate its uptake back into the organism [12]. Siderophores are essential for *A. fumigatus* virulence and deletions of genes involved in siderophore biosynthesis significantly impair fungal virulence [13]. Petrik et al. have taken advantage of the similarity of iron and gallium chemistry, already widely exploited in the use of ^{67}Ga citrate, to label two siderophores with the short-lived, generator-produced isotope ^{68}Ga . They found that both compounds had good in vitro characteristics with respect to stability and specific uptake into *A. fumigatus* in culture as demonstrated by competition by either excess iron or unlabelled siderophore. Using small-animal PET/CT imaging in an immunocompromised rat model of lung infection with *A. fumigatus*,

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they report promising findings with selective accumulation in diseased lung tissue of both compounds and correlation with the severity of the infection. The high stability observed in vitro was maintained in vivo resulting in low uptake in non-target tissues.

As discussed above, the diagnosis of IA is currently in stalemate, where even a so-called diagnostic approach will not actually identify an organism with certainty in many patients. The ability to identify early infection with evidence that the causative agent is *A. fumigatus* would be a significant step forward. Does the work of Petrik et al. meet the necessary requirements to deliver an early and pathogen-specific investigation? With respect to timing, it remains unclear how early IA could be identified by labelled siderophores. Time-course experiments should clarify this in their rat model using a tracheal inoculum of *A. fumigatus*. However, how this correlates with human infection and when to investigate in patients (for persistent fever?) can only be determined by clinical trials. Another key factor is the specificity of labelled siderophores for *A. fumigatus*. There has been an increase in the frequency of other *Aspergillus* species associated with IA, including *A. flavus*, *A. terreus*, *A. nidulans* and *A. niger* [14–16], as well as an increase in non-*Aspergillus* moulds [4]. Consequently, an assay that is truly specific for *A. fumigatus* will give a false-negative result for IA and IFD due to other moulds, while detection of a broad range of moulds will make pathogen identification impossible. Either scenario is important as the management decisions, particularly drug choice, are not the same for all IA caused by other species or for other IFD. Even more importantly, to what extent are these labelled siderophores taken up by bacterial organisms?

Petrik et al. are to be commended in their efforts to develop new techniques for the diagnosis of IA and their ongoing preclinical work will determine if this approach, with the selected compounds and ^{68}Ga , will progress to studies in humans. There is clearly much to be done before this will affect clinical practice, but if labelled siderophores were to provide an early and specific marker of IA in the high-risk immunocompromised patient population, this would represent a breakthrough in management.

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