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ImmunoPET/MR imaging allows specific detection of Aspergillus fumigatus lung infection in vivo.


Comment: Invasive pulmonary aspergillosis (IPA) is a devastating disease with high mortality rates affecting immunocompromised individuals. The condition remains challenging to diagnose hence delaying treatment. Novel approaches to diagnosis that may be clinically applied are required and an active area of ongoing research. In this interesting approach by a German group, a novel probe for the non-invasive detection of A. fumigatus lung infection was developed based on antibody-guided positron emission tomography and magnetic resonance imaging (termed immunoPET/MR). This probe [64Cu]DOTA-JF5 is able to (i) identify specific localization of Aspergillus lung infection by co-localizing with invasive hyphae and, (ii) distinguish IPA from bacterial lung infections and also from general increases in metabolic activity associated with lung inflammation (a function of [18F]FDG-PET). This study is the first where an antibody-guided in vivo imaging system has been applied for non-invasive diagnosis of IPA, a major problem in ICUs worldwide and possessing great potential for clinical translation.

Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management.


Comment: Chronic pulmonary aspergillosis (CPA) is an important Aspergillus-associated pulmonary manifestation in the non-immunocompromised patient that complicates a range of chronic respiratory disorders of high prevalence in Asia such as bronchiectasis. Several forms exist including chronic cavitary pulmonary aspergillosis (CCPA) that if untreated progresses to chronic fibrosing pulmonary aspergillosis (CFPA). Other forms include an aspergilloma (fungal ball), aspergillus nodule or in the moderately immunocompromised, subacute invasive pulmonary aspergillosis (previously termed chronic necrotizing pulmonary aspergillosis). Prior to this publication, very limited information was available for clinicians to provide guidance in the diagnosis and/or management of CPA. A group of experts have convened to produce this excellent publication that provides the first clinical, radiological and microbiological guidelines for this complex and commonly overlooked condition affecting a significant group of our chronic respiratory patients.

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Putative invasive pulmonary aspergillosis in critically ill patients with chronic obstructive pulmonary disease: a matched cohort study.


Comment: Patients with chronic obstructive pulmonary disease (COPD) are at high risks of developing aspergillosis, particularly invasive in view of the recurrent courses of steroids used in exacerbations. This contributes to immunosuppression. These French investigators have derived a clinical algorithm to differentiate colonization from putative invasive pulmonary aspergillosis (PIPA) in sputum positive Aspergillus patients who are critically ill. Using matched cohorts across two university hospitals, they compared n=50 cases of PIPA in critically ill patients with COPD in the ICU with n=100 control patients with COPD. It was found that short and long term mortality was higher in the PIPA group and that PIPA was a strong independent risk factor for death. Prior to ICU admission, use of corticosteroids and/or antibiotics significantly increased risks of PIPA. PIPA was unrelated to a specific bacterial pattern of colonization or infection. Anti-fungal treatment (used in 64% PIPA cases) had minimal impact on outcome. The emergence of Aspergillus associated colonization and disease in COPD is highlighted by this important study that also serves as an important reminder of potential dangers associated with recurrent steroid and antibiotic use in chronic lung disease.

Blood basophil activation is a reliable biomarker of allergic bronchopulmonary aspergillosis in cystic fibrosis.


Comment: The diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis (CF) remains clinically challenging because of overlapping clinical, microbiological, radiological and immunological features of a bacterial exacerbation. To date, no objective biological test exists to aid clinicians in diagnosing ABPA. This collaborative study between American and Irish groups focused on blood basophils as they play a major role in allergic responses. It was discovered that changes in basophil surface activation pattern occurs in ABPA and can discriminate between patients with and without ABPA. Specifically, in a prospective and longitudinal fashion, the investigators show that the basophil activation test (BAT) levels of CD203c, a basophil surface marker can reliably discriminate between ABPA, Non-ABPA and Aspergillus colonization without ABPA. This was confirmed in a validation cohort providing for the first time a potential diagnostic and stable monitoring marker of ABPA in CF.
A randomised trial of glucocorticoids in acute-stage allergic bronchopulmonary aspergillosis complicating asthma.


Comment: It remains unclear whether the use of high-dose steroid therapy in the treatment of acute allergic bronchopulmonary aspergillosis (ABPA) results in better long-term outcomes. This solid study from India compared the safety and efficacy of two glucocorticoid protocols in ABPA in treatment-naïve ABPA patients. Recruited subjects received either the medium dose steroid protocol (defined as oral prednisolone 0.5 mg/kg/day for 2 weeks followed by 0.5 mg/kg on alternate days for 8 weeks, then taper by 5 mg every 2 weeks and discontinue after 3–5 months) or the high dose steroid protocol (oral prednisolone 0.75 mg/kg/day for 6 weeks followed by 0.5 mg/kg/day for 6 weeks, then taper by 5 mg every 6 weeks and discontinue after 8–10 months). Primary outcome was exacerbation rates and the presence of steroid-dependent disease after 1 and 2 years of treatment.

Secondary end-points for the study included composite response rates after 6 weeks, improvement in lung function, time to first exacerbation, cumulative dose and adverse effects of steroid treatment. N=92 subjects were studied (medium dose, n=48 and high dose, n=44). No differences were detected in the primary endpoint between the two groups illustrating that medium dose oral glucocorticoids are as effective and safer than high-dose for ABPA treatment. Of note, while composite response rates were significantly higher in the high-dose group, no differences in lung function improvement and/or time to first exacerbation were observed between groups. Cumulative glucocorticoid dose and side-effects were as expected significantly higher in the high-dose group. This important study provides clinicians a basis for use of medium dose steroids in the treatment of acute ABPA.

The basophil surface marker CD203c identifies Aspergillus species sensitization in patients with cystic fibrosis.


Comment: Colonization by Aspergillus fumigatus in patients with cystic fibrosis (CF) can cause sensitization, an entity that likely precedes allergic bronchopulmonary aspergillosis (ABPA) and independently has negative effects on pulmonary function and clinical outcomes. Like ABPA, Aspergillus sensitization is challenging to diagnose. This Irish study assessed the usefulness of the Basophil activation test (BAT) in identifying patients with Aspergillus sensitization. BAT measures CD203c levels by flow cytometry, a basophil surface marker that is upregulated on exposure to specific allergens to which an individual is sensitized.
The authors found that BAT testing discriminates Aspergillus sensitized from non-sensitized patients and that recurrent isolation of Aspergillus from sputum is a significant risk factor for Aspergillus sensitization. Importantly, combining BAT testing with routine serological testing (total IgE and Aspergillus-specific IgE) clearly differentiates CF patients into three groups: non-sensitized, A. fumigatus-sensitized and ABPA. This paper significantly advances the application of diagnostics for Aspergillus-associated disease states in CF however will likely translate to and have application in other respiratory disease states such as asthma and chronic obstructive pulmonary disease (COPD).

**Evolution of the Immune Response to Chronic Airway Colonization with Aspergillus fumigatus Hyphae.**


Comment: Airway colonization with Aspergillus fumigatus is common in patients with underlying chronic lung disease however it’s clinical impact and the need for eradication in this setting is less clear. Studies to probe the inflammatory response to the presence of the fungi in the airway have been hampered by the lack of an animal model of chronic colonization in the setting of immunocompetence. This Canadian study establishes robustly an in-vivo model to study the natural history of airway colonization with A. fumigatus. This was achieved by infecting mice intratracheally with conidia embedded in agar beads. Aspergillus hyphae are shown to exit the beads and persist in the lungs for up to 28 days without invasive disease. Subsequently, the group examined the evolution of the immune response to airway Aspergillus colonization for the first time and discovered that in the initial (early) phase, fungal lesions in the airway incite a robust neutrophilic inflammatory reaction with peribronchial infiltration of lymphocytes and high burden of pro-inflammatory cytokines and chemokines. Evidence of a Th2 response was also observed early in the course of colonization together with increases in IL-4, systemic IgE and airway hyperresponsiveness. This is an important key first report of the evolution of the immune response to airway A. fumigatus colonization states suggestive of the negative effects of the presence of airway Aspergillus even in the absence of allergic or invasive manifestations.

**Prevalence and mechanism of triazole resistance in Aspergillus fumigatus in a referral chest hospital in Delhi, India and an update of the situation in Asia.**


Comment: While Aspergillus fumigatus can cause a range of Aspergillus-associated pulmonary diseases, the mainstay of treatment remains triazole therapy. Global concerns have been raised about the
emergence of triazole resistance which represents a global threat and will complicate existing treatment protocols for aspergillosis syndromes. This study from India reports the prevalence of resistance in A. fumigatus isolates over a four-year period and reviews the current state of this important and emerging issue in Asia and the Middle East. Aspergillus species (n=2117, 45.4% A. flavus; 32.4% A. fumigatus, 15.6% A. species and 6.6% A. terreus) were screened for azole resistance. Azole resistance was detected in n=12 (1.7%) of A. isolates. 83.3% (n=10) of these triazole resistant A. fumigatus (TRAF) isolates with a pan-azole resistant phenotype harbored a TR34/L98H mutation. Among the other two TRAF isolates, one had a G54E and the other three non-synonymous point mutations. Patients from which these isolates were derived included all forms of Aspergillus-associated pulmonary disease including allergic bronchopulmonary syndrome, chronic pulmonary aspergillosis and invasive disease. Interestingly, the Indian TR34/L98H isolates had a unique phenotype that was distinct from similar Chinese, Middle East and European strains. This identified resistance mechanism is linked to fungicide use in agricultural practices in Europe as the isolates are mainly derived from azole naïve patients. From an Asian context, these authors suggest that similar environmental resistance mechanisms likely occur in both China and India, two highly populated countries and likely also in the Middle East. This serves a reminder of the emerging threat from resistance and also of the environmental and agricultural influences of this from a fungal context.

**Validation of a new Aspergillus real-time PCR assay for direct detection of Aspergillus and azole resistance of Aspergillus fumigatus on bronchoalveolar lavage fluid.**


Comment: The diagnosis of pulmonary aspergillosis syndromes continues to pose clinical challenges. Yield from culture is usually poor or late in the course of disease hence increased focus on molecular approaches to airway identification is of interest. This study, a collaboration of Dutch and Belgian researchers validates AsperGenius, a new multiplex real time PCR assay able to identify both clinically relevant Aspergillus species and one that detects resistance (TR34, L98H, T289A, and Y121F mutations in CYP51A) permitting differentiation between susceptible and resistant strains. The authors tested its diagnostic performance on n=37 bronchoalveolar lavage (BAL) fluid samples from hematology patients and n= 40 BAL fluid samples from intensive care unit (ICU) patients using a BAL fluid galactomannan level of ≥1.0 or positive culture as the gold standard for detecting the presence of Aspergillus. In the two groups, n=22 BAL samples were from patients with invasive aspergillosis (IA): 2 proven, 9 probably and 11 non-classifiable. Nineteen (of the 22) were positive according to the gold standard. Sixteen (of the 19) had a positive PCR resulting in a sensitivity, specificity, and positive and negative predictive values of
88.9%, 89.3%, 72.7%, and 96.2%, respectively, for the hematology group and 80.0%, 93.3%, 80.0%, and 93.3%, respectively, in the ICU group. Two resistant strains were identified where voriconazole therapy had failed in both patients. This paper describes an important advance in the field of Aspergillus diagnostics, the AsperGenius multiplex real-time PCR allows for sensitive and rapid detection of Aspergillus species from BAL samples including the identification of resistant strains even where fungal cultures are negative.

**Estimation of the burden of chronic and allergic pulmonary aspergillosis in India.**


**Comment:** While Aspergillus-associated disease is emerging and being recognized in a variety of chronic respiratory disease settings, precise disease burdens particularly of chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA) remain unclear. This study from India estimates the burden of two important Aspergillus-associated disease states: CPA following pulmonary tuberculosis (TB), and ABPA (and severe asthma with fungal sensitization [SAFS]) complicating asthma. Using population estimates for India, the authors estimated an asthma prevalence of 27.6 million. ABPA burden and SAFS were best estimated at 1.38 [range, 0.86-1.52] and 0.96 [range, 0.6-1.06] million respectively. Incident TB cases were approximately 2.1 million with annual incidence of CPA varied at 27,000-0.17 million cases. Where the authors estimated mortality of CPA as 15% annually, the 5-year prevalence of CPA was 290,147 cases with a 5-year prevalence rate of 24 per 100,000. This important study in a large Asian country illustrates the significant burden of ABPA, SAFS and CPA in India. Similar studies are necessary in other Asian countries to best understand and accurately determine the prevalence of these emerging Aspergillus-associated respiratory disease states.

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