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High level of β-(1,3)-D-glucan antigenaemia in cystic fibrosis in the absence of invasive fungal disease

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ABSTRACT

β-(1,3)-D-glucan (BDG) is used to rule out invasive fungal disease (IFD) but its usefulness in cystic fibrosis (CF) has not been evaluated. We measured serum BDG in CF patients with no clinical suspicion of IFD. Samples from 46 adult CF patients during a stable period and during pulmonary exacerbation were tested. The association of BDG with clinical variables was analyzed. Three hundred and three non-CF patients with suspected IFD were used as comparators. Both samples were negative in 52% of CF patients, whereas 67% of comparators had only negative results ($P = 0.08$). CF patients with pancreatic insufficiency and CF-related diabetes had fewer negative results ($P < 0.05$ for both). Negative results were more common in older CF patients ($P < 0.05$). Use of antibiotics, presence of fungi in sputum and CF liver disease did not impact BDG levels. In conclusion, patients with CF experience significant BDG antigenaemia in the absence of IFD.

1. Introduction

Serum β-(1,3)-D-glucan (BDG) testing is recommended by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) for the exclusion of invasive fungal diseases (IFDs) in septic patients who do not respond to antibiotics (Adriaanse et al., 2015). This has been shown to be safe and effective in antifungal stewardship (Alexander et al., 2010; Chotirmall et al., 2010). BDG is a polysaccharide of glucose found in the cell walls of most fungi, including *Aspergillus*, *Candida,* and *Pneumocystis* species. Healthy individuals have very low serum BDG levels (typically 0–40 pg/ml), but in patients with IFD the concentration increases as fungal cells shed BDG into the bloodstream (Cook et al., 1980). IFDs, especially candidaemia, are difficult to treat and are associated with high mortality (Cuenca-Estrella et al., 2012). At the same time, many antifungal drugs have considerable side effects and a high cost, and their overuse is a risk for the development of resistance (De Lisle and Borowitz, 2013). Thus, there is a clear clinical need for quick, reliable and sensitive methods for the detection of fungal infection that can guide antifungal treatment.

The BDG test has high sensitivity and high negative predictive value in low prevalence settings (Digby et al., 2003). It is useful in ruling out candidaemia, aspergillosis and pneumocystosis, but fungal species in the genera *Cryptococcus*, *Blastomyces, Lichtheimia, Mucor,* and *Rhizopus* either lack BDG completely or produce very small amounts and thus may not be detected with this method (Ellis, 2004; van Elburg et al., 1996). Some sources of false positives have also been identified. For example, surgical gauzes have been shown to release BDG and cause false positive serum BDG results (Fillaux et al., 2012). Elevated levels have also been reported in lung transplant recipients, ICU patients, and HIV patients in the absence of IFD (Fuchs et al., 1994; Girouard et al., 2007; Hong et al., 2004). BDG has also been detected in some antimicrobial agents but these are an unlikely cause of false positives due to becoming diluted when distributed in the body (Kataoka et al., 2002). High bilirubin and triglyceride levels may inhibit the BDG assay and cause false-negative results (Digby et al., 2003).

Cystic fibrosis (CF) is a genetic condition caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene that leads to dehydration of fluids in the respiratory and gastrointestinal tracts, causing mucus plugging, blockage of fluid secretion, inflammation, tissue damage and fibrosis. There is growing evidence that fungal pathogens can have a significant impact on the clinical outcome of CF. Chronic colonization of the lungs by *Aspergillus* and *Candida* species has been linked with more rapid decline in lung function and more frequent respiratory exacerbations (de Kort et al., 2011; Kikkert et al., 2007). Patients colonized by *Aspergillus* may also develop allergic bronchopulmonary

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aspergillosis (ABPA), a hypersensitivity reaction to the fungus that is associated with decreased lung function (Marty et al., 2006). Despite our better understanding of fungal respiratory diseases we still know very little about the level of fungal antigenaemia in CF. In addition, permanent intravenous access devices are commonly used in CF due to repeated need for venepuncture and IV antibiotics. Infection of these devices by fungal pathogens is a common cause for their removal.

In our clinical experience, serum BDG test in septic CF patients is often reported positive in the absence of any other evidence of IFD (e.g. negative blood culture, no response to antifungal treatment). To our knowledge, there have been no studies assessing the use of the BDG test in CF patients. Thus, the aim of this study was to determine the level of BDG antigenaemia in adult CF patients who have no clinical suspicion of IFD, both during a period of clinical stability and during a pulmonary exacerbation. We also recorded various clinical variables to assess their relationship with BDG levels in these patients.

2. Methods

2.1. Patients

Paired serum and sputum samples were obtained from the Manchester Allergy, Respiratory and Thoracic Surgery Biobank (ManARTS). Samples collected from 46 CF patients at the Manchester Adult Cystic Fibrosis Centre at University Hospital of South Manchester (UHSM) NHS Trust between 2013 and 2015 were included in the study. The study was approved by the ManARTS ethics committee (REC reference: 10/H1010/7). None of the patients had any clinical suspicion of IFD at either time point and did not receive systemic antifungal therapy. Two pairs of serum and sputum samples were obtained for each patient; one serum and sputum pair taken when the patient was stable, and another pair taken during a respiratory exacerbation (total 92 serum and 92 sputum samples). To further analyze the variability of BDG levels over the 13-month study period, 3–4 additional serum samples (mixture of stable and exacerbation) were analyzed for 3 patients with low BDG values and 3 patients with high BDG values.

Respiratory exacerbation was diagnosed when oral or intravenous antibiotic treatment was prescribed and the patient displayed in the previous 7 days any four of the following symptoms: increased cough, increased dyspnoea, change in sputum, new or increased haemoptysis, malaise, fever >38 °C, anorexia, sinus pain, new physical findings in the chest, fall in spirometry by >10%, new radiographic findings (modified Fuchs criteria) (McIntosh et al., 2005). All patients were over 18 years old and had no history of HIV infection or transplantation, and were able to spontaneously provide sputum. At the time of sampling, the median age of participants was 30.0 years (range 21.1–65.7), and 59% of the patients were male. The median time between the stable and exacerbation samples was 115.5 days (range 22–377). A spectrum of clinical variables was recorded, including paired sputum fungal culture, white cell count (WCC) and C-reactive protein (CRP), CF genotype, nebulised antibiotic use, oral steroid use, lung function tests and presence of pancreatic insufficiency (PI), CF related diabetes (CFRD), and CF liver disease (CFLD). The number of days on intravenous (IV) antibiotics in the year 2014 was also recorded.

2.2. Comparator group

A database search was performed of all serum BDG results for adult patients at UHSM reported by the Mycology Reference Centre Manchester between 2013 and 2015; any results from CF patients were excluded. The total number of comparator patients was 303 with 433 total samples. The median age of comparators at the time of sampling was 59.8 years (range 18.2–90.0), and 63% of the patients were male. At UHSM the BDG test is used to rule out a diagnosis of IFD in septic patients who are non-responsive to broad-spectrum antibiotics and have risk factors for invasive fungal disease. The test is not used for routine screening or to rule in IFD. Thus, all patients in the comparator group had suspected IFD.

2.3. BDG assay

(1,3)-β-D-Glucan concentration was measured using the Fungitell® assay (Associates of Cape Cod, East Falmouth, MA, USA) according to manufacturer's instructions. Sputum samples were diluted 1:100 with glucan-free water, serum samples were not diluted. 5 μl of each sample was pre-treated with 20 μl of the alkaline reagent (final concentration 0.125 M KOH and 0.6 M KCl) at 37 °C for 10 minutes. Five concentrations of glucan standard were prepared (range 31.25–500 pg/ml) and added to the plate along with a negative control. After this, 100 μl of the Fungitell® reagent was added to each well and the plate was kinetically monitored at 405 nm minus 490 nm in an incubating plate reader (37 °C) for 40 minutes. The mean rate of optical density change between 0 and 40 minutes was calculated and BDG concentration determined by using the standard curve. BDG values <60 pg/ml were interpreted as negative (IFD unlikely), 60–79 pg/ml as indeterminate (IFD possible), and ≥80 pg/ml as positive (IFD possible), as per kit instructions. Patients with negative BDG results only were classified as "negative", and those with one or more positive or indeterminate results were classified as "not negative". In addition, a sample of one commonly used short-acting insulin, NovoRapid® Insulin Aspartate (100 units/ml) (Novo Nordisk, Bagsværd, Denmark) was analyzed for BDG.

2.4. Statistical methods

Statistical analysis was performed using SPSS Statistics version 22 (IBM Corporation, Armonk, NY) and Graphpad Prism version 6 (GraphPad Software Inc., San Diego, CA, USA). Stable and exacerbation samples were analyzed separately. BDG values were not normally distributed so non-parametric tests were used for analysis. Spearman rank correlation was used to analyze the association between stable and exacerbation BDG levels, between serum and sputum BDG levels, and between BDG levels and the other continuous variables. Wilcoxon matched pairs test was used to analyze the difference in BDG levels between paired stable and exacerbation samples. Mann–Whitney U-test was used to analyze the association of BDG levels with various categorical variables. The patients were divided into 2 groups based on their serum BDG result ("negative" and "not negative"), and the Mann–Whitney test was used for analyzing the differences in the continuous variables between these 2 groups. Chi-squared test was used to analyze differences in categorical variables between the "negative and "not negative" groups. Chi-squared test was also used to analyze the differences in the proportions of "negative" or "not negative" results between the CF group and the control group. The Kruskall–Wallis test was used for comparing the BDG levels of the 3 different genotype groups. Any comparison of values with a level of significance, $P < 0.05$, was reported as statistically significant.

3. Results

3.1. Serum BDG levels

In the CF group the median serum BDG concentration was 45.7 pg/ml (range 0–472.3 pg/ml). Overall 60% of stable and 59% of exacerbation samples were negative. Both stable and exacerbation serum samples were negative for 24 (52%) of the 46 CF patients, one of the samples was negative for 6 (13%) patients, and for 16 (35%) patients neither of the 2 samples was negative. There was no significant difference between stable and exacerbation serum BDG levels (median 40.2 vs. 48.7 pg/ml respectively, $P = 0.544$), and the levels correlated closely within patients ($r = 0.856$, $P < 0.0001$). In the suspected IFD group, the median serum BDG concentration was 34.3 pg/ml (range 31.3–>500 pg/ml). Overall