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Prognostic potential of 1,3-beta-D-glucan levels in bronchoalveolar lavage fluid samples

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Summary *Objectives:* The objective of this study was to investigate the prognostic potential of 1,3-beta-D-glucan (BDG) testing in bronchoalveolar lavage fluid (BALF) samples.
Methods: A total of 300 BALF samples from 252 patients were investigated for BDG (Fungitell® assay). Prognostic potential of BALF BDG was evaluated by using: i.) Kaplan—Meier analysis, and ii.) multivariable Cox hazard regression analyses.

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Results: BALF BDG levels were found to be significantly higher in samples with *Candida* spp. colonization ($p < 0.001$). A total of 61/252 patients (24.2%) died within 90-days of BALF sampling (18.1% of patients with BALF BDG <200 pg/mL, 32.4% with BALF BDG ≥200 pg/mL). Kaplan–Meier analysis revealed that overall cumulative 90-day mortality was significantly higher in those with BALF BDG levels ≥200 pg/mL when compared to those with levels <200 pg/mL (log-rank $p = 0.006$, Breslow $p = 0.005$ and Tarone–Ware $p = 0.005$). The multivariable Cox regression analysis showed that BALF BDG levels were a strong predictor of 90-day overall mortality, with a hazard ratio of 1.048 (per 100 pg/mL increase of BALF BDG).
Conclusion: False positive BALF BDG results in the presence of *Candida* spp. colonization of the lower respiratory tract may explain the limited diagnostic potential of BALF BDG testing. In contrast, prognostic potential of BALF BDG may be promising.

Introduction

1,3-Beta-D-glucan (BDG) is a cell wall component of most pathogenic fungi including *Aspergillus* and *Candida* spp. that is used as a biomarker for invasive fungal infections (IFIs) diagnosis. In a recent meta-analysis, serum BDG determination revealed a pooled sensitivity of 75% for invasive *Candida* infections.[1] Especially the high negative predictive value, of >95% makes BDG determination a valuable tool for clinicians to rule out suspected IFIs.[2] This is of particular interest as up to two-thirds of patients receiving systemic antifungal therapy have no evidence for IFIs.[3] Discontinuation of empirical antifungal therapy based on negative serum BDG levels was shown to be safe and was not associated with relapsing IFIs in these patients.[4]

In contrast, recent studies have indicated that bronchoalveolar lavage fluid (BALF) BDG testing has very limited diagnostic potential for IFIs, with imperfect sensitivities (71–90%) and low specificities (36–76%).[5–8] The reason for the particularly low specificity of BDG determination in BALF samples remains unclear. A potential explanation might be that *Candida* spp. colonization of the lower respiratory tract (LRT) may cause elevated BDG levels in BALF also in the absence of IFIs.[5–8] Whether or not this is true remains unclear, however.

To date, a handful of studies have evaluated the prognostic potential of serum BDG testing, reporting that serial serum BDG testing may be used for stratification and response assessment of antifungal treatment and may serve as a predictor for mortality.[9–11] *Candida* colonization of the LRT may be another prognostic parameter that may predict not only overall mortality, but also bacterial airway infection and prolonged ventilator dependency.[12–19] In contrast to serum BDG and *Candida* colonization, prognostic potential of BALF BDG testing has not been evaluated to date.

The objective of this study was i.) to investigate the prognostic potential of BDG levels in BALF samples and ii.) to determine whether *Candida* colonization in LRT is associated with elevated BALF BDG levels.

Materials and methods

Data and sample collection for this cohort study were conducted between February 2012 and May 2014 at the University Hospital of Graz, Austria. A total of 300 consecutive BALF samples were collected from 252 patients with mixed underlying diseases undergoing routine bronchoscopy. The decision to perform bronchoscopy was made by the discretion of the treating physicians. Patients and/or samples included in this study have been in part published previously in studies evaluating novel methods for diagnosis of invasive pulmonary aspergillosis.[7,8,20–26]

The analysis of the samples was done in part prospectively and in part retrospectively. Mycological culture was performed prospectively. Conventional culture techniques were applied and performed for all samples immediately after sample collection to analyze fungal growth at the Institute of Hygiene, Microbiology and Environmental Medicine, Graz and at the Microbiology Laboratory, Department of Internal Medicine University Hospital of Graz. All samples were stored after bronchoscopy at −70 °C for retrospective BDG testing, which was completed within 6 months after sample collection at the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz. For BALF BDG testing the commercially available Fungitell® assay (Associates of Cape Cod, Falmouth, MA) was used, adapted for automation on a BCS-XP coagulation analyzer allowing single-sample testing with extended analytical measuring range and fast turnaround time, as described previously.[27]

For statistical analysis, SPSS 22 (SPSS Inc., Chicago, IL) was used. Impact of binominal variables (e.g. *Candida* growth in BALF culture) on mortality rates was calculated using Chi-square test, while impact on BALF BDG levels was calculated with Mann–Whitney U test. Demographic data are displayed as means plus 95% confidence interval (95% CI) or median plus interquartile 25–75 range (IQR), where appropriate.

Survival analyses always used the first BALF sample per patient (i.e. one sample per patient). For **Kaplan–Meier analysis**, samples were assigned to two defined cut-off groups: < 200 pg/ml BDG (group 1), and >200 pg/ml BDG (group 2). The cutoff of 200 pg/mL was chosen using Youdens index using medians of 90-day mortality versus survival, rounded to the nearest increment of 10 (i. 190, 200, 210). Cumulative 90-day survival was assessed for these three groups using the Kaplan–Meier method and the Mantel-Cox log-rank, Breslow, and Tarone–Ware tests. Survival was assessed from BALF sampling until death (if within 90 days). All surviving patients were censored with the day +90 after BALF sampling. To evaluate the impact