Supplementary data

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Bronchoalveolar lavage triacetylfusarinine C (TAFC) determination for diagnosis of invasive pulmonary aspergillosis in patients with hematological malignancies[☆]

KEYWORDS
Siderophores;
Galactomannan;
Lateral flow device;
Antimould treatment;
Antifungal prophylaxis

☆ Original data of this manuscript have been presented at ID Week 2016, New Orleans, USA (poster presentation number 1558; recipient of ID Week Trainee Travel Grant).

Dear Editor,

We read with interest the paper by Fortun and colleagues[1] who found that galactomannan (GM) testing from bronchoalveolar lavage fluid (BALF) is a promising method for detecting invasive pulmonary aspergillosis (IPA) in patients at risk. As an important limitation, sensitivity of BALF GM may decrease in case of administration of antimould prophylaxis or empirical therapy,[2,3] which are commonly used in high-risk patients with underlying hematological malignancies to reduce the incidence of IPA and improve survival. To increase sensitivity, recent studies suggest to combine BALF GM with other diagnostic tests, such as the *Aspergillus* specific Lateral Flow Device Test (LFD), which is yet not commercially available, or BALF PCR, which lacks standardization.[3–5] Improved diagnostic markers that can be used as combination partners with BALF GM are therefore needed.

Triacetylfusarinine C (TAFC) is one of two different secreted siderophores (i.e. low-molecular mass, ferric iron-specific chelators) produced by *Aspergillus fumigatus* to mediate iron acquisition from the host during infection.[6,7] TAFC is a fungal specific molecule that is also produced by a limited number of other moulds (e.g. *Aspergillus nidulans* and *Fusarium graminearum*), but not yeasts, and animal models have revealed promising results for TAFC-mediated diagnosis of IPA.[7,8] Here we evaluated in patients with hematological malignancies whether sensitivity of BALF GM can be increased by combination with TAFC.

A total of 45 BALF samples obtained from 45 patients with underlying hematological malignancies (15 patients with proven or probable IA and 30 controls with no IPA who were each matched 2:1 by age and underlying diseases) were included in this analysis. IPA was classified according to the revised EORTC/MSG criteria with one modification: exclusion of beta-D-Glucan as mycological criterion.[9] BALF samples were obtained between July 2012 and August 2015 at the Medical University Hospital Graz, Austria. GM (Platelia Aspergillus Ag ELISA; Bio-Rad Laboratories, Munich, Germany) and LFD (OLM Diagnostics, Newcastle upon Tyne, UK)[10] were performed prospectively in all samples, as described previously.[10] Samples were thereafter stored at −70 °C and shipped in 2015 on dry ice to the Innsbruck Medical University for retrospective mass spectrometry-mediated TAFC determination.[7] Investigators in Innsbruck were blinded towards IPA classification of the samples. For BALF GM we evaluated two different cut-offs for determining positivity: >0.5 optical density index (ODI), and >1.0 ODI, which has recently been recommended by the FDA in its Guidance on Qualification of Biomarkers. Receiver operating characteristic (ROC) curves analyses were performed, and area under the curve (AUC) values are presented including 95% confidence intervals (CI) for TAFC. The optimal cut-off for discriminating patients with and without IPA was calculated by using the Youdens index. The study adhered to Declaration of Helsinki, 1996, Good Clinical Practice, and was approved by the local ethics committee, Medical University Graz, Austria (EC-number 23–343 ex 10/11). Statistical analysis was performed using SPSS, version 23 (SPSS Inc., Chicago, IL, USA). Negative predictive value (NPV), positive predictive value (PPV), sensitivity and specificity for single biomarkers and combinations were calculated. A p-value of <0.05 was considered as statistically significant.

A total of 44 samples from 15 patients with probable (n = 14) or proven (n = 1) IPA and 29 controls without evidence of IPA were included in the final analysis (one sample had to be excluded because TAFC measurement failed). Demographic characteristics and underlying diseases of the study population are displayed in Table 1. A total of 11/15 (73%) of patients with probable/proven IPA were receiving mould-active antifungal prophylaxis/therapy at the time of the BALF procedure. AUC for TAFC for differentiating probable/proven from no IPA was 0.601 (0.425–0.777, n.s.), and with Youdes Index we determined an optimal cut-off of >1 ng/ml. Performances of TAFC, GM and LFD as well as combinations are depicted in Table 2. Range of TAFC levels that were considered true positives (i.e. measured in patients with IPA) was 1.4–6.2 ng/ml, range of false positive TAFC levels was 1.2–3.3 ng/ml. While sensitivity of BALF GM as a single test was 73% with the 0.5 ODI cut-off, and 53% when using the 1.0 ODI cut-off, sensitivities could be markedly increased when combining GM with either TAFC or LFD. The combination of either TAFC and/or GM resulting positive exhibited sensitivities of 87% (with 0.5 ODI GM cut-off) and 73% (with 1.0 ODI cut-off) with similar results observed for the combination LFD and/or GM. In two IPA patients with negative BALF GM (<0.5 ODI), both TAFC and LFD resulted positive (both samples resulted also positive with *Aspergillus* specific BALF PCR[4,5] which was performed only in a subset of samples), indicating the additional diagnostic value of both, TAFC and the LFD. While only four BALF samples resulted positive for both TAFC and GM, all these samples originated from patients with probable/proven IPA (100% specificity and PPV).

IPA is associated with high morbidity and mortality in hematological malignancy patients, and early detection is essential for optimal therapeutic success. While diagnostic efforts have been perpetually improved, the optimal use of the available diagnostic repertoire is still a matter of debate. This is true in particular in patients receiving mould active prophylaxis which has been shown to reduce sensitivity of GM and other diagnostic tests for IPA.[2] In the

Table 1 Demographic data and underlying diseases of the study population.

	Probable or proven IPA	No evidence for IPA
Number	15	29
Female (n, %)	9 (60)	17 (59)
Age, years (median, IQR)	60 (56–65)	61 (53–69)
Underlying diseases (n, %)		
Acute myeloid leukemia	8 (53)	16 (55)
Multiple myeloma	3 (20)	6 (21)
Acute lymphocytic leukemia	2 (13)	2[7]
Chronic lymphocytic leukemia	1 (7)	2 (7)
Non-hodgkin lymphoma	1 (7)	2 (7)
Myelodysplastic syndrome	–	1 (3)