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Brief Report

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The screening of blood by *Aspergillus* PCR and galactomannan ELISA precedes BAL detection in patients with proven and probable IA

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Abstract

Early detection of *Aspergillus* infection has the potential to facilitate a more effective management of invasive disease. Data from probable/proven cases of invasive aspergillosis (IA) with a positive galactomannan enzyme-linked immunosorbent assay (GM) bronchoalveolar lavage fluid (BALF) was analyzed in respect to serum GM and/or polymerase chain reaction (PCR) screening of blood samples prior to, or concurrent with bronchoscopy. Concurrent serum GM testing is less sensitive than BALF itself. Nevertheless screening of blood using GM or PCR testing detected IA cases earlier (GM: 42% or PCR: 56%), particularly when combined (GM/PCR: 73%). Therefore, regular screening facilitates and improves early detection of IA in patients suffering from acute leukemia.

Key words: bronchoalveolar lavage, galactomannan ELISA, PCR screening, blood, *Aspergillus*.

Invasive aspergillosis (IA) remains a major complication in hematological malignancies and postallogeneic hematopoietic stem cell transplantation. In these patients, IA is one of the most common causes of mortality due to infection. Early detection of *Aspergillus* infection has the potential to facilitate a more effective management of invasive disease. The optimal sample type and diagnostic method is still under debate. Bronchoalveolar lavage fluid (BALF) directly obtained from the focus of infection is often used to confirm a diagnosis, but blood obtained using minimally-invasive procedures facilitates screening to preempt disease and is also regularly used. Previous studies evaluating the performance of galactomannan enzyme-linked immunosorbent assay (GM) detection on both materials, concomitantly sampled, showed superior performance when testing BALF.[1,2] In this study, routine clinical data from high-risk patients undergoing bronchoscopy for diagnostic purposes were analyzed. Most of these patients were suffering from different hematological malignancies (for details see Table 1). Simultaneous GM testing in BALF and serum was performed and correlated to data from GM serum assays or PCR blood testing done prior to bronchoscopy.

In this retrospective analysis, patient data were screened for positive GM testing (Bio-Rad) of BALF, performed as a routine clinical investigation. Twenty-eight high-risk patients with proven/probable IA according to the revised definitions,[3] who underwent bronchoscopy and had concurrent or prior screening with GM, were included from 2005 to 2017. As the threshold for BALF GM testing was increased from 0.5 to 1.0 in the very recently published (December 2019) update of the EORTC/MSG criteria,[4] two cases would be categorized as possible IFD (see Table 2, patients marked by asterisk [UKW2, UKW4]). The performance of GM was analyzed in BALF and serum in parallel as well as in serum obtained from the same patients prior to BAL. For GM an optical density index (ODI) cutoff of 0.5 (serum and BALF) was used. In parallel, PCR testing of blood samples prior to BAL was performed. Screening with GM/PCR up to 1 month prior to bronchoscopy was included. For DNA extraction

Table 1. Demographics including patients with EORTC/MSG diagnosis proven/probable invasive aspergillosis.

Demographic[a]	$n = 28$
No. of males/no. of females	19/9
Age male (mean [range]) (yr)	53.9 (19–76)
Age female (mean [range]) (yr)	48.8 (18–72)
Underlying condition (no.)	
AML	15
Lymphoma	5
ALL	4
AA	2
Other[b]	2

[a]AA, aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia.
[b]Includes one case of immunocytoma and chronic lymphoblastic leukemia.

3 ml of whole blood or 1 ml of serum/plasma was used following European *Aspergillus* PCR Initiative (EAPCRI) compliant methods for the detection of *Aspergillus* DNA.[5–9]

Patient demographics are shown in Table 1. At the time point when BAL was performed or up to 10 days before or after 21 patients received mould-active antifungal therapy (AFT: voriconazole, $n = 14$, AmBisome, $n = 3$, posaconazole, $n = 2$, a combination of AmBisome and voriconazole, $n = 2$). Six patients received no mould-active AFT, and data were not available for one patient. Forty-six percent of patients (13/28) were neutropenic in this time period (Table 2).

Sixteen out of 28 patients with proven/probable IA had concurrent (the same day) BALF and serum testing (Table 2). And 13/16 patients (81%) had a positive BALF GM test result but negative serum testing; 3/16 (19%) had an additional concurrent positive serum GM test. In 26 cases serum GM testing was performed prior to BAL. In 11/26 cases (42%) positive GM serum testing preceded GM BALF results by up to 31 days (median 10 days, range 0–31). Up to three samples were positive (Table 2, patient CAR16; median: two samples). In 25 cases blood PCR testing was performed prior to BAL. Positive blood PCR testing preceded GM BALF results in 14/25 cases (56%; median 11.5

Table 2. Diagnostic results and clinical data of selected probable/proven IA cases.

Code	BAL GM[a]	GM serum[a]	GM pre-serum[b]	Max. pre-serum GM[c]	PCR blood[b]	Max. pre-PCR[c]	GM/PCR combined	Neutropenia[d,e]	Antifungal treatment[e,f]
CAR1	**5.3**	**0.5**	neg	−16	neg (pos + 4)	−16 (2)	neg (pos + 4 PCR)	no	none
CAR2	**5.1**	neg	neg	−10	**pos (−32)**	−10 (3)	**pos**	yes	voriconazole (−5)
CAR3	**4.8**	neg	**0.6 (−31)**	−31 (9)	neg (pos + 4)	−29 (6)	**pos**	no	posaconazole (−10)
CAR4	**5.2**	neg	neg	−20	**pos (−3)**	−20	**pos**	yes	AmBisome (+1)
CAR5	**1.6**	neg	neg	−21 (5)	**pos (−8,−31)**	−21 (4)	**pos**	no	voriconazole (−10)
CAR6	**3.2**	nd	**0.8, 0.7 (−6/−10)**	−10	neg	−10	**pos**	yes	voriconazole (−5)
CAR7	**3.3**	nd	neg	**−2**	**pos (−13)**	−13 (2)	**pos**	no	none
CAR8	**1.2**	nd	neg	**−2**	**pos (−32)**	−32 (6)	**pos**	yes	AmBisome/voriconazole (nd)
CAR9	**0.6**	nd	**0.5, 1.1 (−8/−12)**	−12	**pos (−1)**	−12 (2)	**pos**	no	posaconazole (−7)
CAR10	**0.5**	nd	neg	−31	**pos (−4,−10)**	−32	**pos**	no	voriconazole (−2)
CAR11	**0.5**	nd	**0.5 (−5)**	**−5**	neg	−31 (5)	**pos**	no	voriconazole (0)
CAR12	**5.3**	nd	**1.2, 0.7 (−5/−15)**	−15 (2)	**pos (−5)**	−30 (2)	**pos**	yes	voriconazole (−4)
CAR13	**6.8**	nd	neg	−14	neg (pos + 3)	−14	neg (pos + 3 PCR)	yes	voriconazole (0)
CAR14	**1.4**	nd	**2.7 (−7)**	**−7**	**pos (−7)**	**−7**	**pos**	no	AmBisome/voriconazole (−4)
CAR15	**4.4**	nd	neg (pos + 31)	−31 (6)	**pos (−26)**	−24 (2)	**pos**	no	AmBisome (0)
CAR16	**2.8**	nd	**1.1, 1.4, 1.1 (−3/−4/−10)**	−10	neg (pos + 6)	−10 (3)	**pos**	yes	voriconazole (−10)
CAR17	**0.5**	nd	neg	−8	**pos (−22)**	−22 (2)	**pos**	no	voriconazole (0)
UKW1	**1.7**	neg	neg	−22	neg (pos + 25)	**−6**	neg (pos + 25 PCR)	yes	none
UKW2*	**0.58**	neg	nd		nd			no	none
UKW3	**1.93**	neg	**0.5 (−18)**	−18 (3)	nd		**pos**	no	voriconazole (−10)
UKW4*	**0.81**	neg	neg	−21 (4)	neg	−14 (3)	neg	no	AmBisome (−2)
UKW5	**1.1**	neg	neg	**−4**	neg	**−4**	neg	yes	none
UKW6	**1.86**	neg	nd		nd			no	voriconazole (−3)
UKW7	**0.58**	**0.67**	**0.8/0.8 (−2/−8)**	−23	**pos (−2)**	**−2**	**pos**	no	voriconazole (+/−0)
UKW8	**2.68**	neg	neg	−20 (5)	neg	−20 (5)	neg	yes	voriconazole (−2)
UKW9	**0.83**	neg	neg	−16	neg	−16	neg	yes	voriconazole (−8)
UKW10	**0.73**	**1.28**	**1.3 (+/−0)**	**−4**	**pos (+/−0)**	**−4**	**pos**	yes	nd
UKW11	**10.2**	neg	**1.6/0.8 (−9/−13)**	−20	**pos (−6/−10/−13)**	−20	**pos**	yes	none
% pos	100%	19%	42%		56%		73%		

BAL, bronchoalveolar lavage; GM, galactomannan; PCR, polymerase chain reaction.
[a]GM optical density index (ODI) for concurrent testing.
[b]Values in brackets indicate the day of testing in relation to BAL; "−" before, "+" after.
[c]Negative value indicates the day of earliest available serum sample for GM/PCR testing; regular screening was performed twice weekly; number in brackets indicates available number of samples for irregular screening; bold values indicate sample availability of maximal 7 days before BAL.
[d]Neutropenia as defined by EORTC/MSG criteria 2008.
[e]Within +/− 10 days of BAL.
[f]Values in brackets indicate the day of first treatment in relation to BAL; "−" before, "+" after.
pos, positive at day (X), "−" indicates the day before BAL was performed; (pos + X), positive GM/PCR screening at day X after BAL was performed; neg, negative; nd, not determined; max., maximum;*: patient will be categorized as possible IFD due to EORTC/MSG criteria 2019.

days, range 0–32 days). Similar to GM serum results one patient showed three positive samples (Table 2, UKW11; median: one sample). By combining both blood biomarkers 73% of all cases (19/26) were detected at an earlier time point to BALF testing. When positive biomarkers after bronchoscopy (up to 4 days, Table 2) are considered, screening positivity increased to 81% (21/26). Interestingly, three of the remaining five patients showed biomarker positivity either post BAL (UKW1, d + 25 PCR, d + 37 GM; UKW8, d + 33 PCR) or within an increased screening period (UKW9, d-57/d-61 PCR). The fourth patient (UKW4) showed a borderline GM at day + 27 (OD, 0.47), whereas the last one (UKW5) was only screened starting with day 4. A higher screening positivity rate is possible if the frequency of blood sampling is increased, or the period prior to bronchoscopy is extended. For GM testing, sera taken maximum 7 days before BAL were available for six of 26 patients; seven patients showed irregular sampling scheme meaning that half of the patients had a suboptimal number of serum samples. Seven of these 13 patients (54%) tested negative for GM. A similar picture was seen for PCR testing with even more suboptimal sampling (19 of 25 patients). Eight of 19 were tested negative using PCR (42%).

GM BALF testing is described to be superior to serum GM testing, and this study confirms that for diagnostic confirmation, where a single sample is tested, BALF testing is superior to testing a single contemporaneously drawn serum sample. On the other hand, screening of easily obtainable blood is described in various studies to facilitate detection of probable/proven IA cases.[10–13] This study shows that screening with both blood GM and PCR generates positivity that frequently precede BALF results and can be used as marker for an earlier diagnosis. Beside GM and PCR there are alternative screening methods (including beta-d-glucan, lateral-flow assays, or conventional culture) and other materials to be used, for example, unprocessed sputum, which recently was successfully used to detect *Aspergillus*.[14]

In summary, GM BALF testing was superior to concurrent GM serum and is the preferred specimen for confirming a diagnosis in the absence of screening. In approximately half of the IA cases, GM serum and blood PCR positivity preceded that of BALF, providing an opportunity for an earlier diagnosis of IA when regular testing is performed. By combining both blood biomarkers 73% of all cases (19/26) were detected at an earlier time point to BALF testing. When positive biomarkers after bronchoscopy (up to 4 days, Table 2) are considered, screening positivity increased to 81%. If regular screening is performed in patients at risk of IA, the need for bronchoscopy can be minimized. In the absence of screening, where IA is suspected, testing a BALF is preferential over a single one-off serum.

Declaration of interest

P.L.W. served on an advisory board for F2G and Gilead and received speakers honoraria from Pfizer, Gilead, MSD, and BOPA; received funding for travel and meeting attendance from Gilead, Launch Diagnostics, BOPA and Bruker Diagnostics; received research funding from Bruker Diagnostics; received payment from F2G for providing diagnostic services; is a founding member of European Aspergillus PCR initiative. J.L. is a founding member of European Aspergillus PCR initiative. J.S. and H.E. report no conflicts of interest. All of the authors are responsible for the content and the writing of the paper.

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