International Journal of Infectious Diseases 83 (2019) 20–25

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Diagnostic role of polymerase chain reaction in bronchoalveolar lavage fluid for invasive pulmonary aspergillosis in immunocompromised patients – A retrospective cohort study

Emilia Hardak[a,f,**], Eyal Fuchs[a,f,*], Hannah Leskes[d], Yuval Geffen[e], Tsila Zuckerman[c,f], Ilana Oren[b,c,f]

[a] Division of Pulmonary Medicine, Rambam Health Care Campus, Haifa, Israel
[b] Division of Infectious Diseases, Rambam Health Care Campus, Haifa, Israel
[c] Department of Hematology and Bone Marrow Transplantation, Rambam Health Care Campus, Haifa, Israel
[d] Laboratory Division Assuta Ashdod Medical center, Ashdod, Israel
[e] Clinical Microbiology Laboratory, Rambam Health Care Campus, Haifa, Israel
[f] Bruce Rappaport Faculty of Medicine, Technion – Israely Institute of Technology, Haifa, Israel

ARTICLE INFO

Article history:
Received 17 January 2019
Received in revised form 17 March 2019
Accepted 21 March 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
PCR
Bronchoalveolar lavage
Diagnosis
Invasive pulmonary aspergillosis

ABSTRACT

Background: This study aimed to evaluate the diagnostic role of PCR detection of Aspergillus DNA in the broncho-alveolar lavage (BAL) fluid in a large cohort of patients suspected to have invasive pulmonary aspergillosis (IPA).
Methods: Consecutive immunocompromised patients who underwent bronchoscopy with BAL sampling and PCR detection of Aspergillus DNA for the diagnosis of pulmonary infiltrates were included in the study. Galactomannan (GM) antigen testing in BAL and serum and BAL fungal culture were also performed. Patients were classified as having IPA (proven/probable/possible) or no-IPA according to the EORTC/MSG diagnostic criteria.
Results: During 12 years (2005–2016), 1248 bronchoscopies were performed for 1072 patients. 77% had hematological malignancy, of them 40% had AML and 35.6% underwent HSCT. IPA was diagnosed in 531 patients (42.5%), 7-proven, 280-probable and 244-possible. PCR was positive in 266 cases, of them 213 had IPA, indicating a true positive rate of 80% (213/266) and a false positive rate of 20% (53/266). These results establish the diagnostic performance of PCR to have sensitivity of 40%, specificity of 93%, PPV- 80% and NPV-68%. Of 244 patients with possible IPA, 80 had positive PCR. Including PCR in the diagnostic criteria would move 80 cases from the possible group to the probable one. A combination of positive PCR and/or BAL-GM increases sensitivity to 74%, while positivity of both tests elevates PPV to 99.4%.
Conclusions: Inclusion PCR for the detection of Aspergillus-DNA in BAL in the mycological criteria of the EORTC/MSG definitions increases the rate and the certainty of IPA diagnosis.

Summary

PCR for the detection of aspergillus DNA in broncho-alveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis was evaluated retrospectively in 1248 immunocompromised patients, and was found to be a valuable aid for early and accurate diagnosis.

Introduction

Invasive aspergillosis (IA) is a common opportunistic fungal infection, mainly affecting patients with severe and prolonged neutropenia (Chamilos et al., 2006; Patterson et al., 2000; Cornillet et al., 2006; Perkhofer et al., 2010; Kontoyiannis et al., 2017; Kontoyiannis and Bodey, 2002). Early diagnosis of invasive pulmonary aspergillosis (IPA) is notoriously difficult, but crucial for prompt treatment initiation required to improve patient outcome (Cordonnier et al., 2006; Nivoix et al., 2008). The revised

* Corresponding author at: Division of Pulmonary Medicine, Rambam Health Care Campus, P.O. Box 9602, Haifa 31096, Israel.
** Corresponding author at: Division of Pulmonology, Rambam Health Care Campus, 8, Ha'Aliya Street, Haifa 3525408, Israel.
E-mail addresses: e_hardak@rambam.health.gov.il (E. Hardak), e_fuchs@rambam.health.gov.il (E. Fuchs).

https://doi.org/10.1016/j.ijid.2019.03.025

diagnostic criteria for invasive fungal disease issued by EORTC/MSG (De Pauw et al., 2008) are still currently used for infection classification. Proven diagnosis of IPA is rarely established, since histological confirmation in patients at risk is difficult mainly due to concomitant thrombocytopenia. The mycological evidence used for the diagnosis of probable IPA includes traditional microbiological microscopy and culture of a respiratory specimen, along with non-culture-based serological tests, like that of GM antigen in serum and bronchoalveolar lavage (BAL) fluid, as well as β-D-Glucan in serum. According to different series, the use of the above mycological criteria still leaves about 30–70% of all patients treated for IPA, categorized as possible cases (Johnson et al., 2015; Buess et al., 2012; Boch et al., 2016).

Aspergillus DNA detection in BAL fluid using PCR for the diagnosis of IPA was introduced to the microbiological laboratory of our medical center in 2002. The aim of the current study was to evaluate the performance of this test for the diagnosis of IPA, and to assess its added value to other mycological criteria, in a large cohort of immunocompromised patients.

Patients and methods

The study was approved by the Institutional Review Board of the Rambam Health Care Campus, a tertiary care medical center, with approximately 1000 beds. All consecutive immunocompromised patients with pulmonary infiltrates, who underwent diagnostic fiber-optic bronchoscopy with BAL (FOB-BAL) for the evaluation of the etiology of pulmonary infiltrates between 1/2005-12/2016 and for whom PCR for the detection of aspergillus DNA was performed, were included in the study.

Bronchoscopies were performed using a fiber-optic bronchoscope with cardiopulmonary monitoring. The procedure was conducted under conscious sedation and local anesthesia. The BAL fluid laboratory analysis included the following: cytological staining for the detection of fungal elements, *Pneumocystis jirovecii* (*PJ*) bodies and viral inclusion bodies in alveolar cells; bacterial stains and cultures including specific growth media for *Mycobacterial* spp., *Legionella* spp.; fungal cultures; viral cultures for herpes simplex virus (*HSV),* and cytomegalovirus (*CMV*); PCR for the detection of *Aspergillus* spp., *Legionella* spp., *Mycobacterial* spp., *PJ*, *HSV*, *CMV* and respiratory viruses (influenza, parainfluenza, respiratory syncytial virus, adenovirus, human metapneumovirus) nucleic acid.

The GM antigen in serum and BAL fluid was measured using ELISA according to manufacturer's instructions (PLATELIA *ASPERGILLUS Ag:* Bio-Rad, Marnes-la-Coquette, France). These tests were not performed for all cases. The GM antigen test was defined as positive based on 0.5 ODI cut-off. β-D-Glucan test was not available at our laboratory.

PCR assay for the detection of Aspergillus *sp. DNA*

Total DNA was prepared from 5 ml of a BAL fluid sample using Qiamp DNA mini kit (QIAgen, Hilden. Germany) and was PCR-amplified using a two-step (nested) PCR assay that specifically amplifies a highly conserved Aspergillus species-specific region of the 18S ribosomal RNA gene (Skladny et al., 1999). A 232bp PCR fragment encoded by the human β-globin gene was amplified in parallel as a control for the presence of host DNA (Table 1).

Approximately 50–150 ng of total DNA were amplified in a thermal cycler (T3 Thermocycler, Whatman, Biometra) in the presence of 1.0 U of Taq DNA polymerase (Applied Biosystems, Roche), 6.25 nMol of dNTPs, 20 pMol of each primer in a total volume of 25 μl. First round PCR conditions were as follows: 2 min at 94 °C followed by 23 cycles of 40 s at 94 °C, 1 min at 65 °C and 1 min at 72 °C with a final step of 5 min at 72 °C. For the second round (nested) PCR, 2 μl of the 1st round PCR product were amplified as follows: 2 min at 94 °C followed by 35 cycles of 40 s at 94 °C, 1 min at 65 °C, and 1 min at 72 °C, with a final step at 72 °C for 5 min. PCR products were separated on 2% agarose gels, stained with ethidium bromide, and visualized with UV light. The first PCR step results in amplification of a 405-bp fragment, and the second step amplifies an internal fragment of 236 bp. To definitely establish the identity of PCR product, we occasionally sequenced resulting amplicons.

Table 1
Oligonucleotide primer sequences used for the PCR assay.

	Sequence
1st round PCR primers	Forward AFU7S CGGCCCTTAAATAGCCCG
	Reverse AFU7AS GACCGGGT TTGACCAA TTT
2nd round PCR primers	Forward AFU5S AGGGCCAGCGAGTACATCACCTTG
	Reverse AFU8AS TGCCAACTCCCCTGAGCCAG
Control PCR	Forward Gl1 GGT TGGCCAATCTACTCCCAGG
	Reverse Gl2 TGGTCTCCTTAAACCTGTCTTG

Definitions

The total study group was divided into two subgroups. The IPA subgroup included the patients for whom a diagnosis of IPA was made based on EORTC diagnostic criteria, including proven, probable and possible cases, and received anti-fungal treatment based on this diagnosis. The non-IPA subgroup included the rest of the cohort, for whom other infectious or non-infectious diagnoses were made, and who did not receive anti-fungal treatment.

Descriptive statistics in terms of mean, STD and percentiles were performed on the whole parameters in the study. Diagnostic measurements such as sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV), diagnostic odd ratio and confidence interval were calculated. SPPS version 25 was used for statistical analysis.

Results

During the 12-year study period (January 2005 to December 2016), 1072 patients underwent 1248 bronchoscopies with BAL for a suspected opportunistic lung infection. 630/1072 (59%) of the study population were males; median age was 55 (1–90) years.

A total of 966 bronchoscopies were performed in patients with hematological malignancies (77%), the most common one being acute myeloid leukemia (AML) [382/966 (40%)]. Other hematological malignancies in decreasing frequencies were lymphoma, acute lymphoblastic leukemia (ALL), and multiple myeloma (MM). Thirty six percent of the patients (344/966) underwent hematopoietic stem cell transplantation (HSCT), 98/344 (29%) autologous HSCT and 246/344 (71%) allogeneic. One hundred and four (8%) patients had a solid tumor.

IPA was diagnosed in 531 cases [(531/1248 (42.5%)]; in the rest (717/1248 = 57.5%) of the cases other infectious or non-infectious diagnoses were established and these patients constituted the non-IPA group. Of the 531 IPA cases, 7 were diagnosed as proven IPA (1%), 280 cases (53%) – probable [mycological criteria: 171/280 (61%) using BAL GM, 27/280(10%) – serum GM, 28/280 (10%) – BAL culture, 3/280(1%) – BAL cytological staining and 51/280(18%) – test combination], and 244 (46%) – possible, according to EORTC/MSG Consensus Group revised criteria (9). Patient characteristics and underlying immunodeficiency states are presented in Table 2. Primary anti-fungal prophylaxis with anti-mold active drugs was not standard of care at our center during the study period, and only 31 patients out of the study cohort received posaconazole prophylaxis.

Table 2
Patient's demographic characteristics and underlying immunodeficiencies.

	Total N = 1248 (100%)	IPA group N = 531 (100%)	Non IPA group N = 717 (100%)
Gender			
Male	728 (58%)	317 (60%)	411 (57%)
Female	520 (42%)	214 (40%)	306 (43%)
Age y, median (range)	55 (1–90)	55 (1–86)	55 (1–90)
Type of immunodeficiency			
Hematological malignancy	966 (77%)	488 (92%)	478 (67%)
Solid tumor	104 (8%)	22 (4%)	82 (12%)
Steroid treatment	81 (7%)	9 (2%)	72 (10%)
Solid organ transplantation	40 (3%)	6 (1%)	34 (5%)
HIV	17 (1%)	0	17 (2%)
Other	41 (3%)	6 (1%)	35 (5%)
Type of hematological malignancy			
AML	382 (31%)	230 (43%)	152 (21%)
LY	247 (20%)	105 (20%)	142 (20%)
ALL	131 (10%)	68 (13%)	63 (9%)
MM	67 (5%)	28 (5%)	39 (5%)
CLL	50 (4%)	18 (3%)	32 (4%)
CML	6 (0.4%)	1 (0.1%)	5 (1%)
Other	83 (7%)	38 (7%)	45 (7%)
Total HSCT	344 (28%)	158 (30%)	186 (26%)
Allo HSCT	246 (20%)	116 (22%)	130 (18%)
Auto HSCT	98 (8%)	42(8%)	56(8%)

Acute myeloid leukemia (AML); lymphoma (LY); acute lymphocytic leukemia (ALL); multiple myeloma (MM); chronic lymphocytic leukemia (CLL); chronic myeloid leukemia (CML); hematopoietic stem cell transplantation (HSCT); autologous hematopoietic stem cell transplantation (Auto HSCT); allogeneic hematopoietic stem cell transplantation (Allo HSCT); human immunodeficiency virus (HIV).

Diagnostic performance of PCR test for the detection of aspergillus DNA in BAL

PCR for the detection of aspergillus DNA was performed in all the 1248 cases and was positive in 266, 213 of them had IPA (2% proven +60 % probable + 38% possible), indicating a true positive rate of 80% (213/266). Fifty three patients who belonged to the non-IPA group also had a positive PCR test, indicating a false positive rate of 20% (53/266). These results establish the diagnostic performance of the test to have sensitivity of 40%, specificity of 93%, PPV of 80% and NPV of 68%. Assessing PCR performance in the largest and most common at-risk group (966 patients with hematological malignancies) revealed lower sensitivity of 37%, but very high specificity – 96%, leading to PPV of 90% and NPV of 69%. In contrast, in the remaining group, the total cohort excluding hematological malignancies group (1248-966 = 282), sensitivity and NPV were higher (73% and 94%, respectively), but specificity and PPV were much lower (87% and 51%, respectively).

When considering IPA only as proven and probable cases (with possible cases considered non-IPA), the performance of PCR would have a sensitivity of 46%, specificity of 86%, PPV of 50%, and NPV of 84% (Table 3).

IPA was considered possible in 244 patients, meaning with no positive microbiological criterion. PCR was positive in 80 of them. Including positive PCR as a mycological criterion for the diagnosis of probable IPA would change the proportions of proven/probable/possible cases from 1%/53%/46% to 1%/68%/31%.

Diagnostic performance of supplementary mycological tests

In order to compare the accuracy of aspergillus DNA PCR test to the additional validated tests, we examined the yield of the GM antigen test in BAL fluid and serum, as well as Aspergillus culture in BAL fluid (Table 4). As opposed to the PCR tests, GM tests in serum and BAL were performed in only a fraction out of 1248 patients. Fungal culture was performed for all.

A total of 665 BAL fluid samples were tested for GM antigen. Of this subgroup, 376 patients were diagnosed as IPA (proven/probable/possible), and 289 as non-IPA. Two hundred and forty six BAL samples were found to be positive for GM antigen. Of them 215 had IPA and 31 did not (13% false positive). Based on these results, the performance of a GM test in BAL in our cohort showed sensitivity of 57%, specificity of 89%, PPV of 87% and NPV of 62%.

A total of 670 blood tests were performed for the detection of GM antigen in serum. Of this subgroup, 379 patients were diagnosed with IPA (proven/probable/possible), and in 291 the diagnosis was not confirmed. Seventy-one serum samples were positive for GM antigen; 61 of them belonged to the IPA group and 10 – to the non-IPA group (14% false positive). Based on these results, the performance of GM tests in serum in our cohort showed sensitivity of 16%, specificity of 97%, PPV of 86% and NPV of 47%.

All of the 1248 BAL samples were cultured for Aspergillus. Only 57 of them were found to be positive. Fifty-three positive cultures belonged to the IPA (proven/probable/possible) group, while 4/57 (7%) patients with positive culture were not diagnosed with IPA and were considered colonized. Based on these results, the yield of a positive aspergillus culture in BAL, for the diagnosis of IPA, showed sensitivity of 10%, specificity of 99.5%, PPV of 93% and NPV of 60%.

Only 472 patients were evaluated using all four tests, i.e., BAL GM, BAL PCR, BAL culture and serum GM. Of them, 273 patients were diagnosed with IPA (proven/probable/possible). Assessment of test performances in the latter subgroup did not reveal significant difference from the total cohort.

Table 4
Diagnostic performance of all laboratory tests for IPA (proven/probable/possible) diagnosis.

Test	Performance characteristic (%)				Diagnostic odd ratio confidence interval
	Sensitivity	Specificity	PPV	NPV	
BAL PCR	40	93	80	68	8.4 (6.0–11.6)
BAL GM	57	89	87	62	10.9 (7.27–17.0)
Serum GM	16	97	86	47	6.049 (2.95–12.3)
Culture	10	99.5	93	60	26.6 (8.28–85.76)

PPV – positive predictive value; NPV – negative predictive value; GM – galactomannan test; BAL – bronchoalveolar lavage.

Table 3
Diagnostic performance of PCR in BAL for the diagnosis of IPA.

	Performance characteristic (%)			
	Sensitivity	Specificity	PPV	NPV
PCR for IPA (ppp) in the total cohort	40	93	80	68
PCR for IPA (PP) in the total cohort	46	86	50	84
PCR for IPA (ppp) in patients with hematological malignancy	37	96	90	69
PCR for IPA (ppp) in other patients	73	87	51	94

ppp = proven/probable/possible; pp = proven/probable.

Table 5
Diagnostic performance of combinations of laboratory tests for IPA (proven/probable/possible) diagnosis.

Positive test	Performance characteristic (%)				Diagnostic odd ratio confidence interval
	Sensitivity	Specificity	PPV	NPV	
BAL PCR **or** BAL GM	74	75	81	68	8.7 (6.3–12.2)
BAL PCR **or** Serum GM	53	82	80	55	4.9 (3.5–6.9)
BAL PCR **or** culture	43	92	80	68	8.5 (6.2–11.8)
BAL PCR **or** BAL GM **or** Serum GM	81	63	81	64	7.5 (5.2–10.7)
BAL PCR **or** BAL GM **or** Culture	76	74	81	69	9.0 (6.5–12.5)
BAL PCR **or** Serum GM **or** Culture	55	81	80	56	5.1 (3.7–7.1)
BAL GM **or** Serum GM **or** Culture	74	79	87	60	10.3 (6.8–15.5)
BAL PCR **or** BAL GM **or** Serum GM **or** Culture	83	62	81	66	7.8 (5.5–11.3)
BAL PCR **and** BAL GM	20	99.4	96	65	42.7 (15.6–117.1)
BAL PCR **and** BAL GM **and** Serum GM **and** Culture	1	100	100	58	

Table 5 presents the diagnostic performance of various combinations of existing tests. It shows all the combinations of couplets, triplets and quadruplets of tests, if any of them is positive, and if all are positive. For instance, if PCR **or** GM in BAL is positive, the sensitivity increases from 40% (PCR) and 57% (GM) to 74% at the expense of decreasing specificity from 93% and 89% to 75%. If any of the four tests is positive, the sensitivity increases to 83% and the specificity decreases to 62%. The combination of positive PCR **and** positive BAL GM decreases sensitivity to 20% but specificity increases to 99.4%. Addition of another positive test (either blood GM or culture) tremendously reduces sensitivity, while increasing specificity to 100%.

Discussion

The current study has assessed the diagnostic performance of PCR test for the detection of *Aspergillus* spp. DNA in BAL fluid, aiming to improve the diagnosis of IPA in patients at risk.

To the best of our knowledge, this is the largest reported single-center series of consecutive immunocompromised patients who underwent FOB with BAL sampling for suspected opportunistic pulmonary infection. The prevalence of IPA (proven/probable/possible) in this cohort was 42.5% (531/1248). Since publication of the diagnostic criteria of the EORTC (Ascioglu et al., 2002) in 2002, and of the revised criteria in 2008 (De Pauw et al., 2008), great progress has been made in the diagnosis of IPA, mainly due to the addition of the serological test results to the mycological criteria. From this time on, numerous studies have been conducted evaluating the yield of ASP-DNA detection in the diagnosis of IPA (Johnson et al., 2015; Buess et al., 2012; Guegan et al., 2018; Smibert and Slavin, 2017; Eigl et al., 2017; Heng et al., 2014; Hoenigl et al., 2014; Reinwald et al., 2012a), yet this test has not been incorporated into the diagnostic criteria so far, mainly due to lack of standardization.

Our study has demonstrated that the PCR test for the detection of ASP-DNA in BAL fluid has the sensitivity of 40%, specificity of 93%, PPV of 80% and NPV of 68% for the diagnosis of IPA (proven/probable/possible). When considering IPA only as proven and probable cases, the performance of the PCR shows better sensitivity and NPV (46% and 84%, respectively) at the expense of lower specificity and PPV (86% and 50%, respectively). For the group at highest risk, i.e., patients with underlying hematological malignancies, a positive PCR in BAL had a lower sensitivity (37%) and NPV (69%), but higher specificity (96%) and PPV (90%). On the other hand, for non-hematological patients, sensitivity and NPV were much higher (73% and 94% respectively), while specificity and PPV were lower (87% and 51% respectively). The cause for these differences could not be attributed to anti-mold prophylaxis, since only 31 patients received posaconazole prophylaxis. However, many hematological patients at risk started anti-aspergillus treatment based on characteristic CT results 1-3 days before bronchoscopy was eventually performed, and this could be one of the reasons for lower levels of aspergillus biomarkers in this group of patients, as previously described (Reinwald et al., 2012b). Another explanation of the higher sensitivity in non-hematological patients could be related to the fact that hematological patients at risk were vigorously followed for every sign of possible aspergillus infection. In this cohort, blood GM antigen test was performed twice weekly, and early chest CT was done after 3–4 days of fever unresponsive to broad-spectrum antibiotic treatment. In many cases, CT findings showed only a few small nodular infiltrates. This practice allowed IPA diagnosis at a very early stage. At the same time, in non-hematological patients this diagnosis was established only after investigation for “non-resolving pneumonia”, when radiographic findings showed progressive infection with large, sometimes cavitary, infiltrates. One might expect that at this late stage of infection, the load of aspergillus organisms is higher.

The real contribution of the PCR to the diagnosis of IPA was the upgrading of 80 cases from the possible IPA category to the probable one. This would change the distribution of IPA cases in our series from 1%/53%/46% of proven/probable/possible to 1%/68%/31%. In multiple published studies in patients at risk of IPA, those with the possible category constitute the largest group among IPA patients. Based on our 12-year experience presented herein, we suggest that incorporating PCR results into the mycological criteria increases the degree of the diagnosis certainty.

The nested design of the PCR reaction employed in this study probably accounts for the high specificity of the test. This is despite the fact that nested PCR is known to increase the probability of sample contamination. To address this potential caveat, we recommend using two negative controls: the first is controlling for PCR contamination, where water is substituted for the DNA sample; and the second is controlling for contamination during DNA extraction, where a blank sample is processed in toto up to the PCR reaction step. Inhibition controls were also included for each sample and confirmed the reliability of the PCR amplification.

The performance of other already established mycological criteria in our study showed the detection of GM antigen in BAL to be the most sensitive one, with sensitivity of 57%, compared to that of 40% for PCR, although it was associated with lower specificity equating to 89% vs. 93%, respectively. GM antigen in blood and culture of BAL demonstrated the lowest sensitivity of 16% and 10%, respectively, with corresponding highest specificities of 97% and 99.5%. Routine implementation of all four tests (PCR, GM and culture in BAL and GM in blood) for every suspected case of IPA increases the sensitivity to 83%, if any of them is positive, and the specificity rises to >99% for any combination of two or more positive tests.

Numerous studies have evaluated the utility of PCR for the diagnosis of IPA (Johnson et al., 2015; Reinwald et al., 2012b; Avni et al., 2012; Landlinger et al., 2010; Urabe et al., 2017). Results varied widely between the studies and revealed sensitivity ranging from 26% to 100%. The following reasons could potentially account for such variability. Some studies included only patients with AML and HSCT recipients, while others included a large variety of immunocompromised patients. Likewise, some studies incorporated only patients suspected to have IPA, whereas others included patients under screening surveillance as well. A number of studies were limited to PCR testing of BAL fluid only, while certain studies also included blood PCR. Furthermore, the impact of anti-mold active treatment or prophylaxis received by some patients at the time of BAL sampling could not be ruled out. Moreover, different types of PCR tests and cut-offs of GM antigen positivity were used. Four meta-analyses were published, providing independent and statistically sound representation of clinical performance of the PCR test (Avni et al., 2012; Tuon, 2007; Mengoli et al., 2009; Sun et al., 2011). Based on the above studies and meta-analyses, White et al published, on behalf of the Infectious Diseases Society of America, their recommendation to incorporate PCR in the EORTC/MSG definitions (White et al., 2015).

Our study included 1248 patients treated in a single center. All of them were at risk for IPA, underwent chest CT and FOB, and all of them had their BAL fluid tested by the same PCR assay (which is compatible with EAPCRI recommendations (White et al., 2010)) for detection of aspergillus DNA. Indeed, 41.5% of these patients were diagnosed with IPA. Although the PCR at our center did not reach the highest accuracy compared to all similar studies, yet, based on the high prevalence of the disease, the PPV of PCR would be higher. The most sensitive mycological test for IPA diagnosis in the present study was the detection of GM antigen in BAL, with PCR being only second best. Hence, PCR should not replace GM testing, but its addition to the total mycological test battery increases the diagnosis rate of IPA. In our study, this approach actually allowed upgrading 80 patients from the possible IPA category to the probable one.

Currently, two novel POC (point of care) tests, i.e., aspergillus-specific lateral flow device and aspergillus GM lateral flow assay, are being evaluated for their utility for the diagnosis of IPA (Mercier et al., 2019; Heldt et al., 2018). Although their sensitivity is not highest among all biomarkers, their addition to other mycological tests will further increase the degree of the diagnosis certainty, and definitely allow its earlier establishment.

In conclusion, including PCR test for the detection of aspergillus DNA in BAL in the mycological criteria of the EORTC/MSG definitions increases the rate and the certainty of IPA diagnosis.

Funding

None.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval

The study has been approved by our local ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent

Not relevant (Retrospective study of unidentified subjects).

References

Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis 2002;34:7–14.

Avni T, Levy I, Sprecher H, Yahav D, Leibovici L, Paul M. Diagnostic accuracy of PCR alone compared to galactomannan in bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis: a systematic review. J Clin Microbiol 2012;50:3652–8.

Boch T, Spiess B, Cornely OA, Vehreschild JJ, Rath PM, Steinmann J, et al. Diagnosis of invasive fungal infections in haematological patients by combined use of galactomannan, 1,3-β-D-glucan, Aspergillus PCR, multifungal DNA-microarray, and Aspergillus azole resistance PCRs in blood and bronchoalveolar lavage samples: results of a prospective multicentre study. Clin Microbiol Infect 2016;22:862–8.

Buess M, Cathomas G, Halter J, Junker L, Grendelmeier P, Tamm M, et al. Aspergillus-PCR in bronchoalveolar lavage for detection of invasive pulmonary aspergillosis in immunocompromised patients. BMC Infect Dis 2012;12:237.

Chamilos G, Luna M, Lewis RE, Bodey GP, Chemaly R, Tarrand JJ, et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989–2003). Haematologica 2006;91:986–9.

Cordonnier C, Ribaud P, Herbrecht R, Milpied N, Valteau-Couanet D, Morgan C, et al. Prognostic factors for death due to invasive aspergillosis after hematopoietic stem cell transplantation: a 1-year retrospective study of consecutive patients at French transplantation centers. Clin Infect Dis 2006;42:955–63.

Cornillet A, Camus C, Nimubona S, Gandemer V, Tattevin P, Belleguic C, et al. Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. Clin Infect Dis 2006;43:577–84.

De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008;46:1813–21.

Eigl S, Hoenigl M, Spiess B, Heldt S, Prattes J, Neumeister P, et al. Galactomannan testing and Aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. Med Mycol 2017;55:528–34.

Guegan H, Robert-Gangneux F, Camus C, Belaz S, Marchand T, Baldeyrou M, et al. Improving the diagnosis of invasive aspergillosis by the detection of Aspergillus in broncho-alveolar lavage fluid: comparison of non-culture-based assays. J Infect 2018;76:196–205.

Heldt S, Prattes J, Eigl S, Spiess B, Flick H, Rabensteiner J. Diagnosis of invasive aspergillosis in hematological malignancy patients: performance of cytokines, Asp LFD, and Aspergillus PCR in same day blood and bronchoalveolar lavage samples. J Infect 2018;77(September (3)):235–41.

Heng S-C, Chen SC-A, Morrissey CO, Thursky K, Manser RL, De Silva HD, et al. Clinical utility of Aspergillus galactomannan and PCR in bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in patients with haematological malignancies. Diagn Microbiol Infect Dis 2014;79:322–7.

Hoenigl M, Prattes J, Spiess B, Wagner J, Prueller F, Raggam RB, et al. Performance of galactomannan, beta-d-glucan, Aspergillus lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. J Clin Microbiol 2014;52:2039–45.

Johnson GL, Sarker S-J, Nannini F, Ferrini A, Taylor E, Lass-Flörl C, et al. Aspergillus-specific lateral-flow device and real-time PCR testing of bronchoalveolar lavage fluid: a combination biomarker approach for clinical diagnosis of invasive pulmonary aspergillosis. J Clin Microbiol 2015;53:2103–8.

Kontoyiannis DP, Bodey GP. Invasive aspergillosis in 2002: an update. Eur J Clin Microbiol Infect Dis 2002;21:161–72.

Kontoyiannis DP, Selleslag D, Mullane K, Cornely OA, Hope W, Lortholary O, et al. Impact of unresolved neutropenia in patients with neutropenia and invasive aspergillosis: a post hoc analysis of the SECURE trial. J Antimicrob Chemother 2017;73:757–63.

Landlinger C, Preuner S, Bašková L, van Grotel M, Hartwig NG, Dworzak M, et al. Diagnosis of invasive fungal infections by a real-time panfungal PCR assay in immunocompromised pediatric patients. Leukemia 2010;24:2032–8.

Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. Lancet Infect Dis 2009;9:89–96.

Mercier T, Schauwvlieghe A, de Kort E, Dunbar A, Reynders M, Guldentops E, et al. Diagnosing invasive pulmonary aspergillosis in hematology patients: a retrospective multicenter evaluation of a novel lateral flow device. J Clin Microbiol 2019;(January) pii JCM 01913-18.

Nivoix Y, Velten M, Letscher-Bru V, Moghaddam A, Natarajan-Amé S, Fohrer C, et al. Factors associated with overall and attributable mortality in invasive aspergillosis. Clin Infect Dis 2008;47:1176–84.

Patterson TF, Kirkpatrick WR, White M, Hiemenz JW, Wingard JR, Dupont B, et al. Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. I3 Aspergillus study group. Medicine (Baltimore) 2000;79:250–60.

Perkhofer S, Lass-Flörl C, Hell M, Russ G, Krause R, Hönigl M, et al. The Nationwide Austrian Aspergillus Registry: a prospective data collection on epidemiology, therapy and outcome of invasive mould infections in immunocompromised and/or immunosuppressed patients. Int J Antimicrob Agents 2010;36:531–6.

Reinwald M, Spiess B, Heinz WJ, Vehreschild JJ, Lass-Flörl C, Kiehl M, et al. Diagnosing pulmonary aspergillosis in patients with hematological malignancies: a multicenter prospective evaluation of an Aspergillus PCR assay and a galactomannan ELISA in bronchoalveolar lavage samples. Eur J Haematol 2012a;89:120–7.

Reinwald M, Hummel M, Kovalevskaya E, Spiess B, Heinz WJ, Vehreschild JJ, et al. Therapy with antifungals decreases the diagnostic performance of PCR for diagnosing invasive aspergillosis in bronchoalveolar lavage samples of patients with haematological malignancies. J Antimicrob Chemother 2012b;67:2260–7.

Skladny H, Buchheidt D, Baust C, Krieg-Schneider F, Seifarth W, Leib-Mösch C, et al. Specific detection of Aspergillus species in blood and bronchoalveolar lavage samples of immunocompromised patients by two-step PCR. J Clin Microbiol 1999;37:3865–71.

Smibert OC, Slavin MA. Cart before the horse: use of Aspergillus PCR to increase the diagnostic yield from BAL in hematological patients at risk of invasive aspergillosis. Leuk Lymphoma 2017;58:2773–6.

Sun W, Wang K, Gao W, Su X, Qian Q, Lu X, et al. Evaluation of PCR on bronchoalveolar lavage fluid for diagnosis of invasive aspergillosis: a bivariate metaanalysis and systematic review. PLoS One 2011;6:e28467.

Tuon FF. A systematic literature review on the diagnosis of invasive aspergillosis using polymerase chain reaction (PCR) from bronchoalveolar lavage clinical samples. Rev Iberoam Micol 2007;24:89–94.

Urabe N, Sakamoto S, Sano G, Suzuki J, Hebisawa A, Nakamura Y, et al. Usefulness of two aspergillus PCR assays and aspergillus galactomannan and β-D-glucan testing of bronchoalveolar lavage fluid for diagnosis of chronic pulmonary aspergillosis. J Clin Microbiol 2017;55:1738–46.

White PL, Bretagne S, Klingspor L, Melchers WJG, McCulloch E, Schulz B, et al. Aspergillus PCR: one step closer to standardization. J Clin Microbiol 2010;48:1231–40.

White PL, Wingard JR, Bretagne S, Löffler J, Patterson TF, Slavin MA, et al. Aspergillus polymerase chain reaction: systematic review of evidence for clinical use in comparison with antigen testing. Clin Infect Dis 2015;61:1293–303.