Received: 5 March 2019 | Revised: 29 July 2019 | Accepted: 30 July 2019

DOI: 10.1111/myc.12983

ORIGINAL ARTICLE

mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases | WILEY

Aspergillus specific nested PCR from the site of infection is superior to testing concurrent blood samples in immunocompromised patients with suspected invasive aspergillosis

Tobias Boch[1] | Birgit Spiess[1] | Werner Heinz[2] | Oliver A. Cornely[3] | Rainer Schwerdtfeger[4] | Joachim Hahn[5] | Stefan W. Krause[6] | Matthias Duerken[7] | Hartmut Bertz[8] | Stefan Reuter[9] | Michael Kiehl[10] | Bernd Claus[11] | Peter Markus Deckert[12] | Wolf-Karsten Hofmann[1] | Dieter Buchheidt[1] | Mark Reinwald[12]

[1]University Hospital Mannheim, Heidelberg University, Mannheim, Germany

[2]Würzburg University Hospital, Würzburg, Germany

[3]First Department of Internal Medicine and ZKS Köln, BMBF 01KN1106, University Hospital Cologne, Deutsches Zentrum für Infektionsforschung, Cologne, Germany

[4]Helios Klinikum Buch, Berlin, Germany

[5]Regensburg University Hospital, Regensburg, Germany

[6]Erlangen University Hospital, Erlangen, Germany

[7]Department of Pediatric Hematology, Mannheim University Hospital, Mannheim, Germany

[8]Freiburg University Hospital, Freiburg, Germany

[9]Klinikum Leverkusen, Leverkusen, Germany

[10]Klinikum Frankfurt/Oder, Frankfurt/Oder, Germany

[11]Ludwigshafen General Hospital, Ludwigshafen, Germany

[12]Brandenburg Medical School Theodor Fontane, Brandenburg an der Havel, Germany

Correspondence
Mark Reinwald, Department of Hematology and Oncology, Brandenburg Medical School Theodor Fontane, Brandenburg an der Havel, Germany.
Email: m.reinwald@klinikum-brandenburg.de

Summary

Invasive aspergillosis (IA) is a severe complication in immunocompromised patients. Early diagnosis is crucial to decrease its high mortality, yet the diagnostic gold standard (histopathology and culture) is time-consuming and cannot offer early confirmation of IA. Detection of IA by polymerase chain reaction (PCR) shows promising potential. Various studies have analysed its diagnostic performance in different clinical settings, especially addressing optimal specimen selection. However, direct comparison of different types of specimens in individual patients though essential, is rarely reported. We systematically assessed the diagnostic performance of an *Aspergillus*-specific nested PCR by investigating specimens from the site of infection and comparing it with concurrent blood samples in individual patients (pts) with IA. In a retrospective multicenter analysis PCR was performed on clinical specimens (n = 138) of immunocompromised high-risk pts (n = 133) from the site of infection together with concurrent blood samples.

38 pts were classified as proven/probable, 67 as possible and 28 as no IA according to 2008 European Organization for Research and Treatment of Cancer/Mycoses Study Group consensus definitions. A considerably superior performance of PCR from the site of infection was observed particularly in pts during antifungal prophylaxis (AFP)/antifungal therapy (AFT). Besides a specificity of 85%, sensitivity varied markedly in BAL (64%), CSF (100%), tissue samples (67%) as opposed to concurrent blood samples (8%). Our results further emphasise the need for investigating clinical samples from the site of infection in case of suspected IA to further establish or rule out the diagnosis.

KEYWORDS

antifungal, aspergillosis, *Aspergillus*, BAL, blood, cerebrospinal fluid, comparison, PCR

1 | INTRODUCTION

Invasive aspergillosis (IA) is a severe, life-threatening complication in immunocompromised patients (pts), contributing significantly to morbidity and mortality.[1] Definitive diagnosis of IA remains challenging as present diagnostic standards provide suboptimal accuracy.[2] *Aspergillus* spp. are the most frequent fungal pathogen in haematological high risk pts and invasive pulmonary aspergillosis is the most common cause of mortality due to mould disease.[3] The highest incidence (10% to 20%) and mortality rates (60% to 90%) of IA have been reported following allogeneic hematopoietic stem cell transplantation (HSCT) and heart or lung transplantation.[4,5]

IA typically represents a localised infection with the respiratory tract being the main site of infection[6] leading to invasive pulmonary aspergillosis (IPA). Early diagnosis is crucial to decrease the high mortality,[7] but distinct proof of infection is only defined by the diagnostic gold standard comprising of histopathology and culture which is time consuming and cannot offer early confirmation of IA in the vast majority of cases. As a result, IA is underdiagnosed and, in most cases, antifungal therapeutic efforts are initiated empirically merely based on clinical criteria, e.g. suspicious lung infiltrates on chest computed tomography (CT) scans. The limitations of this approach have been depicted in a study that found only around 50% of pts with CT based IA diagnosis to truly harbour IA after surgical removal of suspected tissue.[8]

This diagnostic dilemma consequently leads to overtreatment and high healthcare costs.[9] Sensitivity of culture-based methods has been reported to be as low as 30%[10] and its results are often not congruent with histological results.[11] There is an urgent need for research into diagnostic tools capable of detecting infection before disease manifests. However, the impaired clinical condition of haematological pts at risk for IA often prohibits invasive diagnostic procedures (eg tissue sampling). Thus, the search for improved diagnostic tools has focused on biomarkers such as Galactomannan (GM), Beta-D-Glucan (BDG), *Aspergillus*-specific polymerase chain reaction (PCR) or the lateral flow device (LFD) (reviewed in[12]). Detection of microorganism specific nucleic acids by PCR has demonstrated promising potential for diagnosing infectious diseases, particularly those where conventional culture methods are less effective. Various *Aspergillus*-specific PCR assays have been described and evaluated in clinical studies.[13,14] PCR-based assays can detect and identify *Aspergillus* spp. rapidly and with reliable results in a variety of clinical samples, eg bronchoalveolar (BAL) fluid,[15] blood,[16] cerebrospinal fluid (CSF),[17] tissue samples[18] or pleural effusions (PE).[19] Several studies have, however, demonstrated the negative impact of prior antifungal treatment or prophylaxis on diagnostic test performance. As a diagnostic test BAL PCR may be less affected by prior antifungal treatment compared to concurrent blood testing,[20] which may be attributed to the higher fungal burden observed at the site of infection.[21] Besides methodological differences, selection of specimens is another decisive aspect in the context of optimised fungal diagnostics. There is currently limited data that explicitly focuses on the direct comparison of an *Aspergillus* specific PCR in blood and in samples from the site of infection from immunocompromised pts harvested at the same time. The significance of PCR assays for detection of fungal pathogens is rising and it will supposedly find its way into the upcoming new EORTC/MSG criteria and is already included in the recent ECCMID Guidelines.[22]

In this context we further investigated the diagnostic potential of an established *Aspergillus* PCR assay for diagnosis of IA with special regard to optimal type of specimen and prior antifungal treatment. We directly compared the diagnostic performance of an Aspergillus specific PCR in multiple specimens representing the direct site of infection (BAL, CSF, tissue) with same-day blood samples (obtained within 24 h) from 133 individual pts.

2 | PATIENTS AND METHODS

The study was approved by the local Ethics Committee (Ethics Committee of the Faculty of Medicine, University of Heidelberg, Germany) and samples were obtained according to good clinical practice guidelines (GCP) in compliance with the Declaration of Helsinki. Due to the retrospective nature of the study protocol to retrospectively investigate the clinical data of patients treated at their own local institutions in a scientific intent and data were obtained anonymised concurrently, the need for written informed consent was waived according to German Ethics Committees regulations.[23]

The study included 133 pts at risk for IA presenting with early signs of suspected IA and for whom 138 samples from the sites of infection

(in five patients different infectious episodes) as well as concurrent blood samples (ie taken within 24 h) were obtained and subjected to PCR analysis and contained within out data bank: 103 BAL samples (of 102 pts), 20 CSF samples (from 18 pts), six tissue samples and nine effusion samples (two collected from two pts at different infectious episodes), collected from October 1995 until August 2013.

The pts were treated in 14 german medical centres: University Hospitals of Mannheim, Wuerzburg, Cologne, Erlangen, Ulm, Freiburg, Heidelberg and Düsseldorf; Bone Marrow Transplantation Center Wiesbaden; and General Hospitals of Ludwigshafen, Passau, Frankfurt/Oder, Bielefeld and Trier.

Patient records were screened for pts for whom PCR results from a clinical specimen from a potential site of infection (eg BAL, CSF, tissue, pleural effusion) as well as from concurrent blood samples, obtained within 24 h, were available. Analyses were performed according to Good Clinical Practice (GCP) guidelines as well as in concordance with the Declaration of Helsinki. The study was approved by the local Ethics Committee (Ethics Committee of the Faculty of Medicine, University of Heidelberg, Germany; Reference Number 2007-240N-MA).

2.1 | Patients characteristics

The majority of pts suffered from malignant haematological diseases. Six pts suffered from solid tumours with consecutive therapy-associated intensive neutropaenia. Twenty-five patients received allogeneic and two patients received autologous stem cell transplantation. Patient characteristics are summarised in Table 1. Infections were classified as proven, probable, possible and no IA according to the 2008 European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) consensus definitions.[24]

2.2 | Underlying antifungal therapy (AFT)

Seventy-six percent of the patients were under mould-active antifungal prophylaxis (AFP) or therapy (AFT) at the time of diagnostic procedures. Patients must have received a full daily dosage of AFT prior to sampling in order to be counted as having received AFT. The median number of antifungal regimes prior to specimen sampling was 1 (range 0-3).

2.3 | Radiological diagnostics

Patients received computed tomography (CT) or magnetic resonance imaging (MRI) scans of organs suspected to harbour IA according to standardised techniques which were analysed by local radiologists at the different centres. Radiological results were interpreted according to 2008 EORTC/MSG criteria and not on the basis of minor radiological signs.

TABLE 1 Patient characteristics

Characteristics	Proven/Probable IFI[a] (n = 38)	Possible IA[a] (n = 67)	No IA (n = 28)
Age (median, range)	54 (2-73)	55 (8-76)	55 (20-76)
Gender (female/male)	11/27	30/37	8/20
Underlying disease			
AML	15	34	3
ALL	4	8	2
NHL	11	17	7
HD	1	-	5
MDS/MPN	-	6	3
Aplastic anaemia	2	-	1
Solid tumour	-	1	5
Other	5	1	2
Among them patients after/with			
Allo-HSCT	8	18	4
Auto-HSCT	1	1	0

Note: Other: Liver cirrhosis, Severe combined immunodeficiency syndrome, chronic polyarthritis with continuous severe steroid treatment, autoimmunehemolytic anaemia with chronic intensive steroid treatment, chronic obstructive pulmonary disease with chronic intensive steroid treatment, polymyalgia rheumatica with intensive immunosuppression (MTX +steroids), solid organ transplantation.

Abbreviations: ALL, acute lymphoblastic leukemia; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; Auto-HSCT, autologous hematopoietic stem cell transplantation; MDS, Myelodysplastic Syndrome; MPN, Myeloproliferative neoplasia; NHL, Non-Hodgkin's-Lymphoma.

[a]According to 2008 EORTC/MSG Criteria.

2.4 | Clinical samples

All diagnostic interventions and sampling were performed due to clinical indication and not solely for study reasons. Peripheral blood samples were collected during routine phlebotomy, in a sterile tube to a final concentration of 1.6 mg EDTA per ml blood. The sample volume was 5 to 7 mL.

2.5 | Bronchoalveolar lavage

Bronchoscopy with BAL was performed as described elsewhere [8], and BAL samples were obtained in a sterile tube without conservation media. The mean sample volume was 10 mL.

2.6 | Cerebrospinal fluid

CSF sampling was performed as clinically indicated; reasons for CSF sampling were suspected CNS infection or suspicion of meningeal involvement or CNS manifestation of the underlying disease. Vials of 1 to 2 mL of CSF were shipped immediately with delivery time of <24 h and the specimens were sent at ambient temperature. The diagnostic work-up was usually performed according to the guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). [20], [21] Typical CSF volumes were between 1-2 mL.

2.7 | Tissue samples

Sampling of tissue effusions was performed as clinically indicated; reasons for sampling were suspected infection or suspected manifestation of the underlying disease. The samples were shipped immediately, with typical delivery time of <24 h. The specimens were sent at ambient temperature and were freshly subjected to PCR analysis. The diagnostic work-up was done according to the local standards of the participating centres, usually according to the guidelines of the Infectious Diseases Working Party of the German Society for Hematology and Oncology

2.8 | Same day (ie concurrent) blood samples

Blood samples included in this trial had to be drawn within 24 h relative to the sampling from the infectious site in order to be included in this study. For patients with repeated blood sampling the frequency of serial blood sampling was between 1-6 days for all cases according to clinical data record sheets.

2.9 | Nested *Aspergillus* PCR

Total DNA was extracted from 1.5 mL of the leucocyte pellet of BAL samples and was processed by an experienced technical assistant unin-formed of the clinical data, to a previously described DNA extraction and nested PCR protocol.[25] During the initial implementation process of the nested PCR assay 26 different fungal strains and 18 bacterial culture strains (including three Penicillium strains) were examined with positive signals only in seven *Aspergillus* species (*A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*, *A. clavatus*, *A. versicolor* and *A. nidulans*).

2.10 | Statistical analysis

Patients' data were collected and inserted into a database (Microsoft Excel 2010; Microsoft Software). Statistical analysis was done with GraphPad Prism 5 (Graph Pad Software). Test for significance was done by Mann-Whitney U test for dependent, continuous variables. Comparison of types of specimen was done with Dunn's multiple comparison test. The significance level was set at a two-sided *P*-value of .05. Calculation was based on patients with proven, probable and no IA. Patients with possible IA did not serve as a negative control as the true nature of their infection cannot be elucidated.

3 | RESULTS

A total of 38 pts with proven (n = 9) or probable IA (n = 29), 67 pts with possible and 28 pts with no IA according to EORTC/MSG 2008 consensus criteria were included in this retrospective analysis.

In total, 103 BAL samples, 20 CSF samples, six tissue samples as well as nine effusion samples were examined by *Aspergillus* specific nested PCR. 76% of patients had already been treated with under mould-active antifungal agents either in therapeutic or prophylactic intent for a minimum of 24 h at the time of diagnostic procedure. From each patient we additionally evaluated one same-day serum sample. For 26 pts serial blood samples were available. Diagnostic performance (sensitivity, specificity, negative and positive likelihood ratio and diagnostic odds ratio) of the PCR analyses in BAL, CSF, tissue or effusion samples and concurrent blood samples was evaluated and directly compared.

For determining diagnostic performance proven and probable IA patients (n = 38) served as positive population while patients classified as "noIA" (for whom IA was highly unlikely based on insufficient host factor criteria or compatible radiological findings) (n = 28) were used as negative population.

Results for patients with possible IA (n = 67) were described, but were excluded from the analysis of PCR diagnostic performance.

Sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR) of the PCR in all samples (BAL, CSF and tissue samples) combined was 68% [95%CI 53%-81%]; 100% [95%CI 88%-100%]; >200 (∞); 0.32 and > 200 (∞). In BAL PCR diagnostic performance was sensitivity 63% [95%CI 46%-78%]; specificity 100% [95%CI 82%-100%]; PLR > 200 (∞); NLR 0.37 and DOR > 200 (∞). In CSF PCR diagnostic performance led to sensitivity 100% [95%CI 61^%-100%]; specificity 100% [95%CI 51%-100%]; PLR > 200 (∞); NLR < 0.01 and DOR > 200 (∞). In tissue and effusion samples PCR diagnostic performance

TABLE 2 Diagnostic performance of PCR

Material	Case definition vs NoIA cases	Sensitivity (95% CI)	Specificity (95% CI)	PLR	NLR	DOR
BAL, CSF, effusion and tissue samples combined	Proven and probable (n = 38)	0.68 (95%CI 0.53-0.81)	1.0 (95%CI 0.88-1.0)	>200	0.32	>200 (∞)
BAL cases (n = 103)	Proven and probable (n = 30[a])	0.63 (95%CI 0.46-0.78)	1.0 (95% CI 0.82-1.0)	>200	0.37	>200 (∞)
CSF cases (n = 20)	Proven and probable (n = 6)	1.0 (95%CI 0.61-1.0)	1.0 (95%CI 0.51-1.0)	>200	<0.01	>200 (∞)
Tissue and Effusion cases (n = 15)	Proven and probable (n = 3[a])	0.67 (95%CI 0.21-0.94)	1.0 (95%CI 0.7-1.0)	>200	0.33	>200
Concurrent single blood samples (± 24 h)	Proven and probable (n = 38)	0.08 (95%CI 0.03-0.2)	0.87 (95%CI 0.7-0.95)	0.58	1.07	0.54

Note: Abbreviations: DOR, Diagnostic odds ratio; NLR, Negative likelihood ratio; PLR, Positive likelihood ratio.
[a]One proven patient had both biopsy and BAL samples analysed.

showed sensitivity 67% [95%CI 21%-94%]; specificity 100% [95%CI 70%-100%]; PLR > 200 (∞); NLR 0.33 and DOR > 200 (∞).

For the corresponding concurrent blood samples PCR diagnostic parameters showed a sensitivity 8% [95%CI 3%-20%]; specificity 87% [95%CI 70%-95%]; PLR 0.58; NLR 1.07 and DOR 0.53. Results of the statistical analysis are summarised in Table 2.

Comparison of PCR sensitivity in each type of sample is depicted in Figure 1 and significance is compared by Dunn's Multiple Comparison Test. PCR in BAL and PCR in CSF were significantly more sensitive compared to PCR in the concurrent corresponding blood samples ($P < .005$).

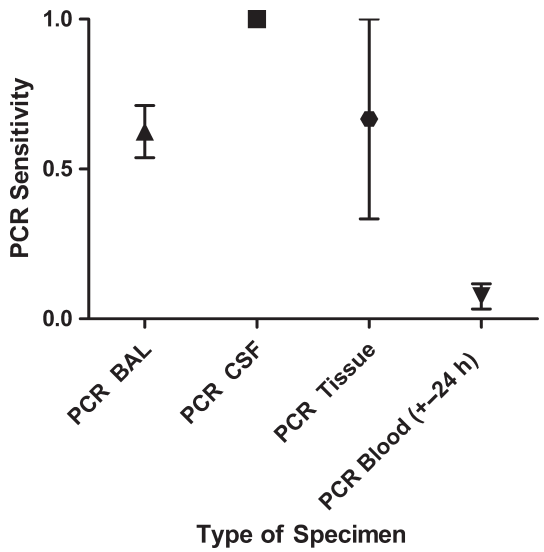

FIGURE 1 Comparison of PCR sensitivity of analysed specimens. Displayed is sensitivity of *Aspergillus*-specific PCR in patients defined as suffering from proven and probable Aspergillosis in different types of specimens, such as Bronchoalveolar lavage (BAL; n = 103 cases), cerebrospinal fluid (CSF; n = 20 cases); Tissue and effusions samples (n = 15 cases) and corresponding blood samples from the same time point. Sensitivity of *Aspergillus*-specific PCR is significantly higher in samples from the site of infection

3.1 | Positivity of *Aspergillus* PCR in patients classified as having possible IA

In patients classified as having possible IA positivity of PCR from the site of the infection was observed in 15/67 cases (in 13 BAL samples and two csf samples). For patients classified as possible positivity of blood PCR was observed in 8/67 cases.

3.2 | Influence of mould active antifungal treatment

In proven and probable IA patients 76% received at least one dosage of mould-active antifungal treatment prior to sampling. Sensitivity of Aspergillus PCR from the site of infection for patients with underlying AFT was 64% compared to 8% for same day blood samples. Proven/probable IA patients having received more than one antifungal (n = 6) prior to sampling showed a further decrease in sensitivity of 50% for BAL PCR. For patients without underlying AFT sensitivity was 75% for samples from the site of the infection compared to 12% from a single concurrent blood sample.

3.3 | Repeated blood sampling and testing

Data on serial blood PCR testing was available for 25 patients. Analysing serial blood samples led to an increase in blood PCR sensitivity (15% [95%CI 7%-29%]), but was still significantly inferior to PCR sensitivity in specimens from the site of infection ($P < .01$). Figure 2 displays different sensitivity values of PCR in specimens from the site of infection, in concurrent blood samples and in serial blood samples if positivity was defined by at least one positive blood PCR result.

3.4 | Results for galactomannan and culture

For 26 proven and probable IA patients galactomannan data were available. Galactomannan either in serum, csf or BAL was positive in 18/26 patients leading to a sensitivity of 69% for GM.

In 21 proven and probable IA patients culture was positive, with 20/21 patients showing growth of *Aspergillus fumigatus* and 1 patient having growth of *Aspergillus nidulans*.

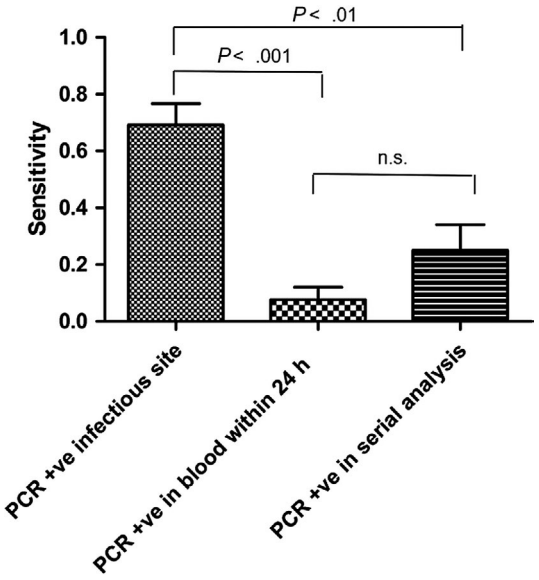

FIGURE 2 Comparison of PCR sensitivity from the site of infectious disease with blood within 24 hrs and repeated sampling. Displayed is sensitivity of *Aspergillus*-specific PCR of samples from the site of infection combined (Bronchoalveolar lavage, cerebrospinal fluid, tissue and effusion samples) in comparison to concurrent blood samples and repeated blood sampling if positivity in blood was defined by at least one positive blood PCR. Sensitivity of blood PCR increases with repeated blood sampling but is still inferior to analysing samples from the site of infection

4 | DISCUSSION

Although Aspergillus-specific PCR has been widely utilised in clinical practice for patients suspected for IA, there is still ongoing debate on the optimal type of specimen, especially as sampling from the site of the infection (such as BAL) is more invasive and potentially associated with a higher risk than analysing blood samples.

Our data demonstrate that the diagnostic performance of PCR is significantly higher in samples representing the infectious site, in most cases BAL. Sensitivity of the PCR in all samples from the site of the infection combined (BAL, CSF, tissue samples) was 70% and thus strikingly higher than in concurrent blood samples obtained directly before or after the diagnostic procedure (sensitivity 8%), but in line with other studies.[26] By obtaining same day blood samples confounding factors such as differences in stage of disease or type and duration of antifungal therapy are equally adjusted. The observed impaired sensitivity biomarkers such as PCR from blood specimens compared to concurrent BAL from the same patients has recently been demonstrated and had been attributed to the high prevalence of underlying AFT and single blood testing in a recent trial.[27]

Furthermore, other studies such as the AMBILOAD Trial [28] have resulted in similar values for diagnostic performance of IA PCR in blood samples. The main reason for the poor diagnostic performance of the blood PCR is most likely previous antifungal treatment or prophylaxis, which has been shown to greatly impair blood PCR performance.[15,16] In our study 76% of the patients were under mould-active antifungal prophylaxis (AFP) or therapy (AFT) at the time of diagnostic procedures. This high rate is due to common clinical practice nowadays, particularly in the context of acute leukaemia as shown by survey data.[29] The results of our study further outline this susceptibility of blood PCR to antifungal treatment and direct comparison illustrates that PCR in BAL, but also in CSF or tissue samples seems to be less affected by AFT, very likely due to higher fungal burden at the site of the infection, which is also confirmed in studies with preclinical animal models.[30] The sensitivity values we observed of 60 to 70% are comparable to other studies including patients under AFT, but are nevertheless worse compared to most of the results obtained from untreated patient cohorts.[31] In addition, many studies addressing the issue of fungal diagnostics are hampered by low numbers of pts with proven or probable IA.[32] Among the patients included within this study were 38 patients classified as proven/probable during the study period of more than 10 years which is an ample number with regard to other studies.

There has been an ongoing debate about the significance and feasibility of bronchoscopy with BAL in heavily immunocompromised patients with lung infiltrates and suspected IPA. Some clinicians tend to avoid performing bronchoscopy because this intervention is not without risk and cannot always be performed when required. Blood samples in various forms (whole blood, plasma or serum) on the other hand are readily available.[7,33,34] However, recent, prospective, multicenter data clearly argues for diagnostic algorithms including BAL in haematological patients with lung infiltrates while the rate of complications was very low.[2,35]

Besides sensitivity, general specificity of the PCR in all different types of samples (including blood) was high and in the case of CSF approaching 100%. Nevertheless, the resulting diagnostic odds ratio and NLR for the blood PCR were not convincing and indicate obvious limitations and lacking diagnostic value of a single blood PCR result. In contrast with the data from our study Springer et al[16] achieved markedly better results for their in-house blood PCR especially for those patients without AFT. However, the significance of blood PCR as either a screening tool or a confirmatory test depends on the population in which it is used: Test performance is heavily influenced by a number of variables such as type and extent of prophylactic or therapeutic antifungal agents,[16,20] prevalence of disease, population analysed or different availability of protective environments.[36]

An essential advantage of the blood PCR over the more invasive BAL, CSF or tissue samples is the possibility of easy serial sampling. We therefore also analysed the diagnostic performance of the blood PCR in the context of repetitive sampling which has been shown to confer higher sensitivity, at least for GM.[37] Repeated blood sampling nearly doubled sensitivity to 15% but was still significantly inferior to PCR in BAL, CSF or tissue samples, underlining the importance of testing samples from the site of infection, an advantage that even cannot be abrogated by repeated blood sampling.

There are some limitations to discuss: (a) The number of proven /probable IA patients (n = 38) seems rather low considering the long time period included yet is in line with other studies dealing with Aspergillus PCR diagnostic performance[27,32] and meta-analysis data

demonstrate number of proven /probable IA patients in publications dealing with Aspergillus PCR performance ranging from 3 to 46[38]; (b) Only for 25 patients serial blood sampling data were available so that the benefit of serial blood testing might be greater than demonstrated in our study.

In conclusion, this study thus demonstrated a significantly higher diagnostic performance of PCR in samples from the site of infection when directly compared to same-day PCR in blood samples of individual patients at risk of IPA especially under antifungal therapy or prophylaxis. Our study thus further reinforces the concept that IA diagnostics should include direct sampling from the site of infection if clinically feasible, in particular for those patients receiving mould-active antifungals.

CONFLICT OF INTEREST

Dr. Boch declares no conflicts of interest. Dr. Spiess declares no conflicts of interest. Dr. Heinz reports research grants from Merck and Pfizer; serves on the speakers bureaus of Alexion, Astellas, Bristol-Myers Squibb, Chugai Pharma, Gilead, Janssen, MSD/Merck and Pfizer; and received travel grants from Alexion, Astellas, MSD/Merck, Novartis and Pfizer. Prof. Cornely has received research grants from Actelion, Amplyx, Astellas, Basilea, Cidara, Da Volterra, F2G, Gilead, Janssen Pharmaceuticals, Medicines Company, MedPace, Melinta Therapeutics, Merck/MSD, Pfizer, Scynexis, is a consultant to Actelion, Allecra Therapeutics, Amplyx, Astellas, Basilea, Biosys UK Limited, Cidara, Da Volterra, Entasis, F2G, Gilead, Matinas, MedPace, Menarini Ricerche, Merck/MSD, Octapharma, Paratek Pharmaceuticals, Pfizer, PSI, Rempex, Scynexis, Seres Therapeutics, Tetraphase, Vical, and received lecture honoraria from Astellas, Basilea, Gilead, Grupo Biotoscana, Merck/MSD and Pfizer. Dr. Schwerdtfeger declares no conflict of interest. Dr. Hahn reports serving on the speakers bureaus of MSD and Basilea Pharma and received travel grants from Gilead. Prof. Krause declares no conflicts of interest. Dr. Duerken declares no conflicts of interest. Prof. Bertz declares no conflicts of interest. Prof. Reuter declares no conflicts of interest. Prof.Kiehl declares no conflicts of interest. Dr. Clauss declares no conflicts of interest. Prof. Deckert declares no conflicts of interest. Prof.Hofmann declares no conflicts of interest. Prof. Buchheidt declares no conflicts of interest. Dr. Reinwald reports on Travel grants from Merck/MSD, Roche and Astellas and research grants from Gilead Sciences and Pfizer and served on the speakers' bureau of Bristol-Myers-Squibb.

AUTHOR CONTRIBUTIONS

TB, DB and MR conceived the study; TB, WH, BS, OC, RS, JH, SK, MD, HB, SR, MK, BC, PMD, WKH, DB and MR collected the data; TB, DB and MR analysed the data; TB and MR led the writing.

ORCID

Werner Heinz https://orcid.org/0000-0002-7833-9202

Oliver A. Cornely https://orcid.org/0000-0001-9599-3137

Mark Reinwald https://orcid.org/0000-0002-6506-2136

REFERENCES

1. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis*. 2010;50(8):1101-1111.
2. Maschmeyer G, Carratala J, Buchheidt D, et al. Diagnosis and antimicrobial therapy of lung infiltrates in febrile neutropenic patients (allogeneic SCT excluded): updated guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Medical Oncology (DGHO). *Ann Oncol*. 2015;26(1):21-33.
3. Patterson TF, Thompson GR 3rd, Denning DW, et al. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016;63(4):e1-e60.
4. Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis*. 2010;50(8):1091-1100.
5. Koehler P, Hamprecht A, Bader O, et al. Epidemiology of invasive aspergillosis and azole resistance in patients with acute leukaemia: the SEPIA Study. *Int J Antimicrob Agents*. 2017;49(2):218-223.
6. Steinbach WJ, Marr KA, Anaissie EJ, et al. Clinical epidemiology of 960 patients with invasive aspergillosis from the PATH Alliance registry. *J Infect*. 2012;65(5):453-464.
7. Shannon VR, Andersson BS, Lei X, Champlin RE, Kontoyiannis DP. Utility of early versus late fiberoptic bronchoscopy in the evaluation of new pulmonary infiltrates following hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2010;45(4):647-655.
8. Kim K, Lee MH, Kim J, et al. Importance of open lung biopsy in the diagnosis of invasive pulmonary aspergillosis in patients with hematologic malignancies. *Am J Hematol*. 2002;71(2):75-79.
9. Ceesay MM, Sadique Z, Harris R, Ehrlich A, Adams EJ, Pagliuca A. Prospective evaluation of the cost of diagnosis and treatment of invasive fungal disease in a cohort of adult haematology patients in the UK. *J Antimicrob Chemother*. 2015;70(4):1175-1181.
10. Tarrand JJ, Lichterfeld M, Warraich I, et al. Diagnosis of invasive septate mold infections. A correlation of microbiological culture and histologic or cytologic examination. *Am J Clin Pathol*. 2003;119(6):854-858.
11. Shah AA, Hazen KC. Diagnostic accuracy of histopathologic and cytopathologic examination of Aspergillus species. *Am J Clin Pathol*. 2013;139(1):55-61.
12. Buchheidt D, Reinwald M, Hoenigl M, Hofmann WK, Spiess B, Boch T. The evolving landscape of new diagnostic tests for invasive aspergillosis in hematology patients: strengths and weaknesses. *Curr Opin Infect Dis*. 2017;30(6):539-544.
13. 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(2):89-96.
14. Heng SC, Chen SC, Morrissey CO, 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(3):322-327.
15. Reinwald M, Hummel M, Kovalevskaya E, 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*. 2012;67(9):2260-2267.
16. Springer J, Lackner M, Nachbaur D, et al. Prospective multicentre PCR-based Aspergillus DNA screening in high-risk patients with and without primary antifungal mould prophylaxis. *Clin Microbiol Infect*. 2016;22(1):80-86.
17. Reinwald M, Buchheidt D, Hummel M, et al. Diagnostic performance of an Aspergillus-specific nested PCR assay in cerebrospinal fluid samples of immunocompromised patients for detection of central nervous system aspergillosis. *PLoS ONE*. 2013;8(2):e56706.
18. Lass-Florl C, Aigner M, Nachbaur D, et al. Diagnosing filamentous fungal infections in immunocompromised patients applying computed tomography-guided percutaneous lung biopsies: a 12-year experience. *Infection*. 2017;45(6):867-875.
19. Bonatti H, Lass-Floerl C, Angerer K, et al. Successful management of postpneumonectomy Aspergillus pleural empyema by combined surgical and anti-fungal treatment with voriconazole and caspofungin. *Mycoses*. 2010;53(5):448-454.
20. Lass-Florl C, Gunsilius E, Gastl G, et al. Diagnosing invasive aspergillosis during antifungal therapy by PCR analysis of blood samples. *J Clin Microbiol*. 2004;42(9):4154-4157.
21. Lengerova M, Kocmanova I, Racil Z, et al. Detection and measurement of fungal burden in a guinea pig model of invasive pulmonary aspergillosis by novel quantitative nested real-time PCR compared with galactomannan and (1,3)-beta-D-glucan detection. *J Clin Microbiol*. 2012;50(3):602-608.
22. Ullmann AJ, Aguado JM, Arikan-Akdagli S, et al. Diagnosis and management of Aspergillus diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. *Clin Microbiol Infect*. 2018;24 (Suppl 1):e1-e38.
23. Raspe HHA, Strech D, Taupitz J. Empfehlungen zur Begutachtung klinischer Studien durch Ethik-Komissionen.: Koelln: Deutscher Aerzte Verlag, 2012.
24. De Pauw B, Walsh TJ, Donnelly JP, 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(12):1813-1821.
25. Skladny H, Buchheidt D, Baust 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(12):3865-3871.
26. 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(2):89-94.
27. Heldt S, Prattes J, Eigl S, et al. 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(3):235-241.
28. Hummel M, Spiess B, Cornely OA, Dittmer M, Morz H, Buchheidt D. Aspergillus PCR testing: results from a prospective PCR study within the AmBiLoad trial. *Eur J Haematol*. 2010;85(2):164-169.
29. van Hal SJ, Gilroy NM, Morrissey CO, et al. Survey of antifungal prophylaxis and fungal diagnostic tests employed in malignant haematology and haemopoietic stem cell transplantation (HSCT) in Australia and New Zealand. *Intern Med J*. 2014;44(12b):1277-1282.
30. Petraitiene R, Petraitis V, Bacher JD, Finkelman MA, Walsh TJ. Effects of host response and antifungal therapy on serum and BAL levels of galactomannan and (1–>3)-beta-D-glucan in experimental invasive pulmonary aspergillosis. *Med Mycol*. 2015;53(6):558-568.
31. Buchheidt D, Baust C, Skladny H, et al. Detection of Aspergillus species in blood and bronchoalveolar lavage samples from immunocompromised patients by means of 2-step polymerase chain reaction: clinical results. *Clin Infect Dis*. 2001;33(4):428-435.
32. Eigl S, Hoenigl M, Spiess B, et al. Galactomannan testing and Aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. *Med Mycol*. 2017;55(5):528-534.
33. Sampsonas F, Kontoyiannis DP, Dickey BF, Evans SE. Performance of a standardized bronchoalveolar lavage protocol in a comprehensive cancer center: a prospective 2-year study. *Cancer*. 2011;117(15):3424-3433.
34. Hummel M, Rudert S, Hof H, Hehlmann R, Buchheidt D. Diagnostic yield of bronchoscopy with bronchoalveolar lavage in febrile patients with hematologic malignancies and pulmonary infiltrates. *Ann Hematol*. 2007;87:291-297.
35. Ruhnke M, Behre G, Buchheidt D, et al. Diagnosis of invasive fungal diseases in haematology and oncology: 2018 update of the recommendations of the infectious diseases working party of the German society for hematology and medical oncology (AGIHO). *Mycoses*. 2018;61:796-813.
36. Barnes RA, White PL, Morton CO, et al. Diagnosis of aspergillosis by PCR: clinical considerations and technical tips. *Med Mycol*. 2018;56(Suppl_1):60-72.
37. Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood*. 2001;97(6):1604-1610.
38. Sun W, Wang K, Gao W, et al. Evaluation of PCR on bronchoalveolar lavage fluid for diagnosis of invasive aspergillosis: a bivariate meta-analysis and systematic review. *PLoS ONE*. 2011;6(12):e28467.

How to cite this article: Boch T, Spiess B, Heinz W, et al. *Aspergillus* specific nested PCR from the site of infection is superior to testing concurrent blood samples in immunocompromised patients with suspected invasive aspergillosis. *Mycoses*. 2019;62:1035–1042. https://doi.org/10.1111/myc.12983