Infection
https://doi.org/10.1007/s15010-018-1224-3

ORIGINAL PAPER

Serological biomarkers of candidemia: a retrospective evaluation of three assays

Karl Dichtl[1] · Ulrich Seybold[2] · Johannes Wagener[1,3,4]

Received: 23 August 2018 / Accepted: 19 September 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Purpose Serologic testing allows for rapid detection of candidemia. More data are needed for the Virion\Serion ELISA antigen test (Ag), Hemkit *Candida* IHA antibody test (Ab), and Wako β-1,3-D-glucan assay (BDG).

Methods Tests were performed on serum samples from 120 cases of culture-confirmed candidemia and 44 *Candida*-negative controls. Sensitivities and specificities of individual tests as well as combinations were assessed.

Results The overall sensitivity of Ag, Ab, and Ag/Ab testing was 30, 40, and 54%, respectively, while in transplant patients it significantly dropped to 16, 26, and 40% ($p = 0.02$). For BDG testing it was 67%, both overall and in transplant patients. Especially Ag testing performed poorly among women ≤ 65 years with a significantly reduced sensitivity of 9% ($p < 0.002$). While the sensitivity of Ag/Ab testing was somewhat higher at 67% for *C. albicans*, it was significantly lower for non-*albicans* species at 42% ($p = 0.006$). The sensitivity of BDG testing for *C. albicans* and non-*albicans* species was not significantly different at 64 and 69%, respectively. Both Ag/Ab and BDG testing had a high specificity of 93%, for Ag testing it was 100%. Similar sensitivities were calculated for sera sampled on the day of and 4–6 days before sampling of positive blood cultures.

Conclusions Serological markers are valuable tools for the early diagnosis of candidemia. Ab, Ag, and BDG testing are all characterized by high specificity. The Wako BDG test is significantly more sensitive compared to combined *Candida*-Ag/Ab testing, particularly in the setting of non-*albicans* species and specific host factors.

Keywords Candidemia · Beta-D-glucan · BDG · Mannan · Serology

Introduction

Increasing incidence of candidemia and in particular of nosocomial bloodstream infections has been observed over the recent years [1–3]. Candidemia is a life-threatening condition associated with a high attributable mortality [4, 5]. Since the outcome depends on early and targeted treatment ("hit hard and early"), prompt diagnosis is essential [4, 6].

Cultivation from blood samples remains the reference method for the detection of candidemia [7, 8]. Compared to bacterial pathogens, blood culture (BC) for *Candida* spp. typically suffer from a lower sensitivity (approx. 50%) and longer time to positivity (TTP) [9]. Serologic tests for invasive fungal diseases allow for a short turnaround time. Hence, testing for biomarkers from blood specimens is recommended when candidemia is suspected [7]. Cell wall constituents are common target structures for antigen detection assays. The widely used Platelia *Candida* serology (Bio-Rad Laboratories, Marnes-la-Coquette, France) is based on two tests that employ cell wall mannan glycoproteins [10]: The *Candida* antigen Plus ELISA is based on the monoclonal anti-mannan antibody EBCA-1 to detect the mannan glycoproteins in serum, and the *Candida* antibody Plus ELISA detects human antibodies that bind to *C. albicans* cell wall mannans. The combination of these two tests for the detection of *Candida* antigen (Ag) and antibodies (Ab) has been

✉ Johannes Wagener
j.wagener@hygiene.uni-wuerzburg.de

1 Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, Medizinische Fakultät, LMU München, Munich, Germany

2 Sektion Klinische Infektiologie, Medizinische Klinik und Poliklinik IV, Klinikum der Universität, LMU Munich, Munich, Germany

3 Institut für Hygiene und Mikrobiologie, Julius-Maximilians-Universität Würzburg, Josef-Schneider-Str. 2/E1, 97080 Würzburg, Germany

4 National Reference Center for Invasive Fungal Infections (NRZMyk), Jena, Germany

Published online: 27 September 2018

extensively studied and is the basis for recommendations in current guidelines [7, 8]. While mannans are specific for *Candida*, β-1,3-D-glucan (BDG) is a general constituent of the cell wall of ascomycete fungi. BDG can be detected in the blood of patients suffering from infections such as invasive candidiasis, invasive aspergillosis, or *P. jirovecii* pneumonia. Although several BDG assays are commercially available, only the FDA-approved and CE-marked Fungitell BDG assay (Associates of Cape Cod, East Falmouth, MA) is commonly used in European and North American laboratories. Consequently, most current studies and guidelines rely on data obtained with the Fungitell BDG assay [7, 8]. However, the usage of this test is confined by methodological and economic challenges [11].

Studies analyzing the performance of serologic tests other than the Platelia and the Fungitell systems are scarce. No published data are available for the Hemkit *Candida* IHA (Ravo Diagnostika) and only two studies with small patient cohorts (21 and 14 cases of invasive candidiasis, respectively) evaluated the Serion ELISA antigen *Candida* kit (Institut Virion\Serion) [12, 13]. For several years, the Wako BDG assay (FUJIFILM Wako Pure Chemical Corporation) was only available in Japan. Very recently, this test has been CE marked and is now available in the Western Hemisphere. To better characterize these three serologic tests, we retrospectively evaluated and compared the Hemkit *Candida* Ab test, the Serion Ag ELISA, and the Wako BDG assay in a clinical cohort comprising 120 episodes of candidemia.

Methods

Study population

This study took place at the Max von Pettenkofer-Institute for Hygiene and Medical Microbiology that hosts the central microbiology laboratory for the University Hospital of Ludwig-Maximilians-University (LMU) Munich, a 2000 bed university medical center in Munich, Germany. Between 2010 and 2017, we identified 120 cases of culture-proven candidemia with corresponding serum samples, which were obtained up to 6 days before sampling of the positive BC. The majority of sera dates from the same day as BC (hereafter referred to as "day 0") (Table 3). We included four patients with two episodes of candidemia representing 7% of all cases. These cases were considered to represent a new episode, since they were characterized by either a temporal distance of ≥3 weeks ($n=2$) or by different species causing the infection ($n=2$). For the *Candida* culture-negative cohort ($n=44$), we included all patients from the period of 2015 to 2017 meeting the following criteria: (1) culture-confirmed bacteremia caused by *Staphylococcus aureus* or *Escherichia coli*; (2) availability of corresponding sera sampled up to 6 days before positive BC. The subgroup of critically ill patients was defined by stay in an intensive care unit. The subgroup of transplant recipients included known recipients of solid organ or bone marrow transplants.

Cultivation and serology

Candida was cultured using the BD BacTec blood culture system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). For the detection of anti-*Candida*-Ab an indirect hemagglutination assay (IHA) was performed at a cutoff titer of 1:320 (Hemkit *Candida* IHA, Ravo Diagnostika, Freiburg, Germany). *Candida* Ag was analysed using the Serion ELISA antigen *Candida*-kit at a cutoff of 2.6 U/ml (Institut Virion\Serion, Würzburg, Germany). BDG measurement was conducted using the Wako BDG assay at a cutoff concentration of 11 pg/ml (FUJIFILM Wako Chemicals Europe, Neuss, Germany). All assays were performed according to the manufacturers' instructions.

Statistical analysis and ethics approval

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). The McNemar's exact test was used for comparison of sensitivities and specificities. Statistical significance was assumed based on an α-level of 0.05. This retrospective study was reviewed and approved by the ethics committee of our university hospital (Ethikkommission der Medizinischen Fakultät der LMU München), and a waiver of informed consent was granted. Sample processing and data analysis were performed anonymously.

Results

Estimation and comparison of test sensitivities

The Ab assay yielded positive results in 48 of 120 cases (40%) (Table 1). The measurements ranged from < 1:80 to > 1:20,480. Using the Ag ELISA, 36 cases (30%) were tested positive. According to the manufacturer's instructions, the Ag ELISA can yield results between defined positivity and negativity. This indeterminate result was reported in five cases (4%) and subsequently analyzed as being negative. Only 19 cases (16%) were tested positive by both the Ab and the Ag assays. Current guidelines recommend the combined use of the Ab and Ag assays [7, 8]. In this study, this combination increased the sensitivity to 54% (65 of 120 cases) (Table 1). The BDG assay demonstrated superior sensitivity compared to the other tests, identifying 80 of 120 (67%) candidemia cases ($p<0.001$ for BDG versus Ag and for BDG versus Ab, $p=0.04$ for BDG versus Ag/Ab). Height

Table 1 Comparison of sensitivities of Ag/Ab- and BDG-based serology

	n	%	Sensitivity (in %)			
			BDG	Ag	Ab	Ag/Ab
Patient conditions						
All patients	120	100	67	30	40	54
Critically ill	66	55	67	33	38	55
Transplant recipients	43	36	67	16	26	40
Patient demographics						
Male	75	63	71	33	39	59
Female	45	38	60	24	42	47
> 65 years	49	41	73	43	51	63
≤ 65 years	71	59	62	21	32	48
Male > 65 years	37	31	73	35	43	59
Female ≤ 65 years	33	28	55	9	30	36
Fungal species						
C. albicans	58	48	64	40	53	67
Non-*C. albicans*	62	52	69	21	27	42
C. glabrata	27	23	67	22	48	59
C. parapsilosis	11	9	73	0	0	0
C. tropicalis	9	8	78	44	22	44
C. krusei	7	6	71	29	14	43
C. guilliermondii	4	3	50	0	25	25
Other *Candida* species	4	3	75	25	25	50

The subgroup of critically ill patients was defined by stay in an intensive care unit. The subgroup of transplant recipients included known recipients of solid organ or bone marrow transplants

BC blood culture, *n* number of cases

and range of measurement results of the different tests are depicted in Fig. 1.

Very recently, Friedrich et al. proposed a cutoff of 3.8 pg/ml for the Wako BDG assay [14]. Applying this cutoff in the present study increased the sensitivity to 81%, but decreased the specificity from 93 to 86%. To evaluate the potential for improved diagnostic accuracy, a receiver operating characteristic (ROC) curve analysis was performed (Fig. 2). The

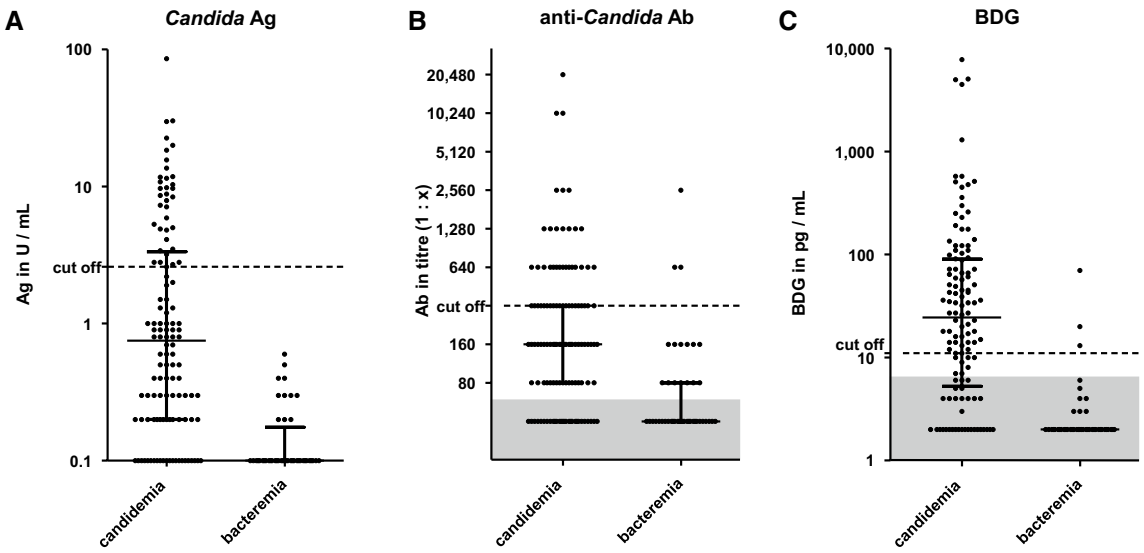

Fig. 1 Box plot (with median and interquartile ranges) of results of **a** Ag (in U/ml), **b** anti-*Candida*-Ab (in titres of 1: dilution factor) and **c** BDG (in pg/ml) assays. Dotted lines indicate the cutoff (11 pg/ml). Results beneath the limit of detection are plotted (not to scale) in the shaded area

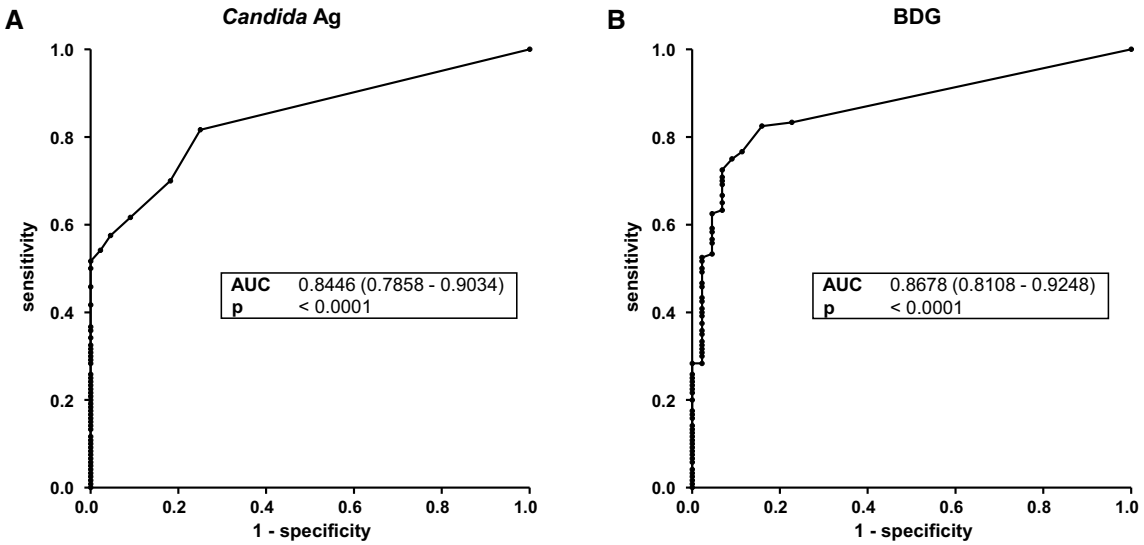

Fig. 2 Receiver operating characteristic (ROC) analysis of **a** *Candida* Ag and **b** BDG for discrimination between candidemia and control patients. Results for the area under the curve (AUC) are specified including the confidence interval (in brackets)

highest Youden's index was found to be 0.71 for a BDG cutoff of 3.95, which is very similar to the cutoff proposed by Friedrich et al. However, according to the manufacturer, the assay's technical limit of detection is 6 pg/ml. By reducing the cutoff from 11 to 7 pg/ml, the sensitivity of BDG testing increased to 73% without any loss of specificity. A sensitivity of 53% and a specificity of 100% were found when a cutoff of 0.57 U/ml was used for the Ag ELISA (Youden's index of 0.57). The combined Ag/Ab testing with the optimized ELISA cutoff identified 83 of 120 cases of candidemia (69%).

Analysis of host and pathogen factors that influence test performance

While sensitivities were not different in the subgroup of critically ill patients, we found a significant decrease when Ag/Ab serology was applied in transplant recipients (40% versus 62% in non-transplanted patients, $p < 0.05$). Interestingly, this cannot be solely attributed to a deficiency in Ab production, but also to a reduced performance of the Ag assay (Table 1). For the individual Ab and Ag detection systems, the respective sensitivities dropped from 40 to 26% and from 30 to 16% in the cohort of transplant recipients. In contrast, the performance of the BDG-based serology was not affected in this subgroup with a sensitivity of 67%. Surprisingly, sensitivities of all tests were lower in the subgroup of female patients ≤ 65 years. Particularly, the Ag ELISA performed poorly, identifying only 9% of cases of candidemia (versus 41% in the residual cases, $p < 0.002$).

In 58 of the 120 cases, only one BC was obtained (Table 2). A set of two BCs sampled on day 0 was available

Table 2 Characteristics of blood cultures sampled at day 0

	n	%	Fraction of pos. BCs (in %)		
			1 pos	2 pos	3 pos
1 BC	58	48	100		
2 BCs	38	32	68	32	
3 BCs	24	20	54	21	25

BC blood culture; *n* number of cases; % percentage of all cases included in the study; *pos*. positive

in 38 cases and a set of three BCs sampled on day 0 in 24 cases (Table 2). Only in the minority of these cases with multiple BCs, all BCs yielded growth of *Candida* (29%). The most frequently isolated species was *C. albicans* (48%), followed by *C. glabrata* (23%), *C. parapsilosis* (9%), *C. tropicalis* (8%), *C. krusei* (6%) and *C. guilliermondii* (3%). Furthermore, single episodes of candidemia were caused by *C. fabianii, C. kefyr, C. lusitaniae* and *C. pelliculosa*. Ag/Ab testing performed significantly better in blood stream infections caused by *C. albicans* than in cases caused by other species (67% versus 42%, $p < 0.01$). Strikingly, Ag/Ab-based serology failed to detect any candidemia caused by *C. parapsilosis*. The different species did not significantly influence the performance of the BDG assay. Particularly in *C.* non-*albicans* blood stream infections, BDG measurement identified significantly more infections than Ag/Ab testing (69% vs. 42%, $p < 0.01$).

The present study includes sera that were collected up to 6 days before the corresponding BC. The specimens were divided into three subgroups depending on the date of sampling: sera obtained simultaneously with the positive BC

(= day 0; 43% of all cases), samples obtained up to 3 days before positive BC (43%) and samples even further predating the positive BC (14%) (Table 3). Notably, we observed no significant differences in sensitivity in these different groups. With respect to this finding, we aimed to evaluate the ability of serologic testing to provide earlier evidence for candidemia compared to BC. We identified 50 cases which met the subsequent criteria. (1) Besides the positive BC, additional BC(s) had been collected, which were negative for *Candida*. (2) The serum was sampled simultaneously with or prior to the negative BC(s). 50% and 58% of these bloodstream infections were tested positive by Ag/Ab and by BDG analysis, respectively.

Estimation of test specificities

To establish a control group, we identified 44 patients suffering from bacteremia with either *S. aureus* (19 cases) or *E. coli* (25 cases) (Table 4). None of the samples were tested positive by the Ag ELISA, but three specimens were tested positive for anti-*Candida*-Ab. Three different samples yielded positive results in the BDG assay. Thus, specificity for both Ag/Ab- and BDG-based serology was 93%.

Discussion

To our knowledge and similar to the recently published study of Friedrich et al., this study is based on the largest cohort of patients who meet the EORTC/MSG criteria for a proven invasive *Candida* infection [14, 15]. The species distribution is in good agreement with previously published surveillance data [16]. Current recommendations and guidelines primarily rely on investigations of the more widespread Fungitell BDG test and the Platelia system [7, 8]. Reviewing 14 studies, which included between 7 and 105 cases of invasive candidiasis, Mikulska and colleagues report a sensitivity of 83% (95% confidence interval, 79–87%) and a specificity of 86% (95% confidence interval, 82–90%) for the Platelia ELISAs [17]. In this study, the combination of the Serion ELISA and the Ravo IHA demonstrated a specificity of 93% and a sensitivity of only 54%. Sensitivity increased to 67% in cases of candidemia caused by *C. albicans*, but decreased to 42% in infections caused by other *Candida* species.

This finding could be explained by the nature of the Ag/Ab assays. The ELISA employs polyclonal antibodies directed against *C. albicans* cell wall Ag and the IHA detects anti-*Candida*-Ab via agglutination with sheep erythrocytes coated with *C. albicans* serotype A cell wall components. However, previous data also suggest a hampered performance of the Platelia kits in the setting of *C.* non-*albicans* and particularly in *C. parapsilosis* infections [18–20]. Applying the Hemkit *Candida* IHA and the Serion antigen ELISA, we also identified a striking loss of performance in this subgroup (sensitivity: 0/11). This is of particular interest since this species accounts for an increasing number of *Candida* infections [21]. Importantly, BDG measurement was not impaired in the setting of *C.* non-*albicans* candidemia.

Very recently, Friedrich et al. reported a low sensitivity of only 43% and a specificity of 98% in the setting of candidemia for the Wako BDG test [14]. In marked contrast, our study demonstrated a much higher sensitivity of 67% and a specificity of 93% in a similar-sized patient cohort. Based on the present data, we cannot explain the discrepant

Table 3 Comparison of Ag/Ab and BDG-based serology with a focus on the course of infection

	n	%	Sensitivity (in %)			
			BDG	Ag	Ab	Ag/Ab
All cases	120	100	67	30	40	54
Time lag to BC						
Same day as BC	52	43	69	40	35	56
1–3 days before BC	51	43	65	41	27	53
4–6 days before BC	17	12	65	24	35	53
Sera prior to negative BC	50	42	58	22	38	50

BC blood culture; *n* number of cases; %, percentage of all cases included in the study

Table 4 Comparison of specificities of Ag/Ab- and BDG-based serology in episodes of bacteremia

	n	%	Specificity (in %)			
			BDG	Ag	Ab	Ag/Ab
Bacteremia	44	100	93	100	93	93
Escherichia coli	25	57	88	100	96	96
Staphylococcus aureus	19	43	100	100	89	89

n number of cases; % percentage of all cases included in the study

results obtained in the two different studies. To improve the sensitivity of the Wako BDG test, Friedrich and colleagues suggested decreasing the cutoff from 11 to 3.8 pg/ml. This increased the sensitivity to 71% [14]. Similarly, when applying a decreased cutoff of 7 pg/ml in our study, we obtained a sensitivity of 73%. Importantly, in both studies, specificities of the Wako BDG test remained as high as 92% (Friedrich et al.) and 93% (our study), even after applying the modified cutoffs. A high specificity is crucial for screening purposes, such as BDG testing twice a week as recommended in the ESCMID (European Society for Clinical Microbiology and Infectious Diseases) guideline for diagnosis and management of *Candida* disease [7]. The most striking effect upon optimization of the cutoff was observed for the Ag ELISA. The sensitivity increased from 30% (2.6 U/ml) to 53% (0.57 U/ml) without loss of specificity. Thereby, combined Ag/Ab testing, as recommended by current guidelines, even has a sensitivity of 69% [7, 8].

Interestingly, previous meta-analyses evaluating the performance of BDG testing for the diagnosis of invasive fungal infections reported sensitivities of 75–80% for BDG testing. However, the increased sensitivities came at a cost of specificity, which was found to range from 82 to 85% [22–24]. The different results reported by the meta-analyses and the present studies by Friedrich et al. and ours are most likely linked to the different test systems evaluated and the applied cutoff values defined by the manufacturers, as discussed above. While most of the earlier work was evaluating the Fungitell assay, the recent work of Friedrich et al., and our work evaluated the Wako BDG test. The results of our study indicate that the BDG assay can be a valuable tool for the clinician to support the diagnosis of candidemia. However, the specificity of 93% limits its use for screening purposes. To improve the positive predictive value, the test could be restricted to patients with a reasonable likelihood for candidemia. Clinical scores such as the *Candida* score for critically ill patients could help to identify the patients at risk for candidemia [25, 26].

Our data reveal an unexpected influence of host factors on the detection of antigenemia. The sensitivity of the Ag ELISA was nearly reduced to half when the test was applied in transplant recipients. In contrast, the sensitivity of the BDG assay was not affected. However, this finding is surprising, since cells of the immune system are commonly supposed to be a major cause of clearance of fungal Ag from the bloodstream. For instance, testing for the *Aspergillus*-specific Ag galactomannan is considered to have the highest sensitivity in immunocompromised and immunosuppressed individuals, i.e., in hematologic patients and particularly in bone marrow transplant recipients [27]. Notably, detection rates of both Ag-based tests, i.e., the Wako BDG assay and the Serion ELISA, were demonstrated to be numerically better (but without reaching statistical significance) in male patients than in female patients (71% and 33% vs. 60% and 24%) and in individuals age ≥ 65 years than in younger individuals (73% and 43% vs. 62% and 21%). This difference was significant in the subgroup of women < 65 years, when the sensitivity of the Ag ELISA decreased to only 9%. To our knowledge, the finding of a gender dependency of an Ag-based assay has not been reported before. The nature of this phenomenon remains to be elucidated.

Diagnosis of candidemia via culture is impaired by two major drawbacks: (1) the significant delay due to incubation until positivity and (2) the low sensitivity of BC of about 50% [9]. The phenomenon of poor BC positivity rates was also observed in the present study. In only 29% of all cases with ≥ 2 BC on day 0, *Candida* was cultivated in all BC samples. Interestingly, in a subgroup of 50 cases, for which sera were available that were sampled prior or simultaneously to at least one BC negative for *Candida*, 50% and 58% of patients were Ag/Ab and BDG seropositive, respectively. Notably, increasing the time interval between sampling of the serum and the positive BC only marginally affected the sensitivity of serology. Furthermore, the TTP of the BC has to be considered, which typically results in a further delay of diagnosis of additional 2–3 days [28]. Consequently, combining BCs with serology will most likely allow for a more rapid and early diagnosis of candidemia which is pivotal for the outcome [29]. To make use of this temporal advantage, rapid processing of the sample is required. For economic reasons, serology laboratories will typically collect specimens for batch runs, especially if ELISAs such as the Fungitell BDG assay are applied [11]. The Ravo Hemkit hemagglutination assay and the Wako BDG test, which were used in this study, are suitable for analysis of single samples and therefore allow for prompt reporting.

It must be considered that the inclusion criterion of a BC positive for *Candida* may confound the results and interpretation of this study. For example, it appears more likely to detect fungal Ag in the blood of patients whose vascular fungal load is high enough to yield growth in BC than in blood where the fungal load is too low to yield positive BCs. For similar reasons, it is not possible to estimate the overall sensitivity of serology in patients with *Candida* blood stream infections. Future studies evaluating the performance of serological biomarkers in culture-negative candidemia are necessary to address this issue.

For all three tests evaluated in this study, the amount of data evaluating their performance is limited. Our results demonstrate that the analysis of serological markers is a valuable tool for the early diagnosis. All tests demonstrated high specificities. The Wako BDG test is characterized by a significantly higher sensitivity compared to the combination of *Candida*-Ag and anti-*Candida*-Ab assays. The latter ones were found to suffer from impaired sensitivity depending on host factors and fungal species. Optimizing the cutoff levels

for BDG and Ag testing may improve the performance of both tests for the diagnosis of candidemia.

Acknowledgements This study was supported by FUJIFILM Wako Chemicals Europe. We thank Hellen Müller for technical support.

Compliance with ethical standards

Conflict of interest The authors report financial support for consumables and staff to conduct the study and temporary supply of technical equipment from FUJIFILM Wako Chemicals Europe. The funding source was not involved in the study design, in the collection, analysis and interpretation of data or in writing of the report.

References

1. Bassetti M, Merelli M, Righi E, Diaz-Martin A, Rosello EM, Luzzati R, et al. Epidemiology, species distribution, antifungal susceptibility, and outcome of candidemia across five sites in Italy and Spain. J Clin Microbiol. 2013;51:4167–72.
2. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20:133–63.
3. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39:309–17.
4. Bassetti M, Righi E, Ansaldi F, Merelli M, Trucchi C, Cecilia T, et al. A multicenter study of septic shock due to candidemia: outcomes and predictors of mortality. Intensive Care Med. 2014;40:839–45.
5. Weiss E, Timsit J-F. Management of invasive candidiasis in non-neutropenic ICU patients. Ther Adv Infect Dis. 2014;2:105–15.
6. Kollef M, Micek S, Hampton N, Doherty JA, Kumar A. Septic shock attributed to Candida infection: importance of empiric therapy and source control. Clin Infect Dis. 2012;54:1739–46.
7. Cuenca-Estrella M, Verweij PE, Arendrup MC, Arikan-Akdagli S, Bille J, Donnelly JP, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: diagnostic procedures. Clin Microbiol Infect. 2012;18:9–18.
8. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;62:e1–50.
9. Clancy CJ, Nguyen MH. Diagnosing invasive candidiasis. J Clin Microbiol. 2018;56:01909.
10. Sendid B, Tabouret M, Poirot JL, Mathieu D, Fruit J, Poulain D. New enzyme immunoassays for sensitive detection of circulating *Candida albicans* mannan and antimannan antibodies: useful combined test for diagnosis of systemic candidiasis. J Clin Microbiol. 1999;37:1510–7.
11. Tran T, Beal SG. Application of the 1,3-β-D-glucan (Fungitell) assay in the diagnosis of invasive fungal infections. Arch Pathol Lab Med. 2016;140:181–5.
12. Debusmann F, Schaffrinski M, Rüchel R. Serologische candidose-diagnostik: vergleich von drei antigentests. Der Mikrobiologe. 2008;18:261–2.
13. Lunel FMV, Mennink-Kersten MASH, Ruegebrink D, van der Lee HAL, Donnelly JP, Blijlevens NMA, et al. Value of Candida serum markers in patients with invasive candidiasis after myeloablative chemotherapy. Diagn Microbiol Infect Dis. 2009;64:408–15.
14. Friedrich R, Rappold E, Bogdan C, Held J. Comparative analysis of the wako β-glucan test and the fungitell® assay for the diagnosis of candidemia and pneumocystis jirovecii pneumonia. J Clin Microbiol. 2018. https://doi.org/10.1128/JCM.00464-18.
15. 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.
16. Arendrup MC, Bruun B, Christensen JJ, Fuursted K, Johansen HK, Kjaeldgaard P, et al. National surveillance of fungemia in Denmark (2004 to 2009). J Clin Microbiol. 2011;49:325–34.
17. Mikulska M, Calandra T, Sanguinetti M, Poulain D, Viscoli C. Third European Conference on Infections in Leukemia Group. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. Crit Care. 2010;14:R222.
18. Sendid B, Poirot JL, Tabouret M, Bonnin A, Caillot D, Camus D, et al. Combined detection of mannanaemia and antimannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic Candida species. J Med Microbiol. 2002;51:433–42.
19. Yera H, Sendid B, Francois N, Camus D, Poulain D. Contribution of serological tests and blood culture to the early diagnosis of systemic candidiasis. Eur J Clin Microbiol Infect Dis. 2001;20:864–70.
20. Held J, Kohlberger I, Rappold E, Busse Grawitz A, Häcker G. Comparison of (1->3)-β-D-glucan, mannan/anti-mannan antibodies, and Cand-Tec Candida antigen as serum biomarkers for candidemia. J Clin Microbiol. 2013;51:1158–64.
21. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. *Candida glabrata, Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev. 2012;36:288–305.
22. Lu Y, Chen Y-Q, Guo Y-L, Qin S-M, Wu C, Wang K. Diagnosis of invasive fungal disease using serum (1→3)-β-D-glucan: a bivariate meta-analysis. Intern Med. 2011;50:2783–91.
23. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, et al. Diagnostic accuracy of serum 1,3-β-D-glucan for pneumocystis jiroveci pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J Clin Microbiol. 2012;50:7–15.
24. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. β-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clin Infect Dis. 2011;52:750–70.
25. Kratzer C, Graninger W, Lassnigg A, Presterl E. Design and use of Candida scores at the intensive care unit. Mycoses. 2011;54:467–74.
26. León C, Ruiz-Santana S, Saavedra P, Almirante B, Nolla-Salas J, Alvarez-Lerma F, et al. A bedside scoring system ("Candida score") for early antifungal treatment in nonneutropenic critically ill patients with Candida colonization. Crit Care Med. 2006;34:730–7.
27. Ku NS, Han SH, Choi JY, Kim SB, Kim H-W, Jeong SJ, et al. Diagnostic value of the serum galactomannan assay for invasive aspergillosis: it is less useful in non-haematological patients. Scand J Infect Dis. 2012;44:600–4.
28. Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve

understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013;56:1284–92.
29. Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH, et al. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. Clin Infect Dis. 2012;54:1110–22.