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Lower beta-1,3-D-glucan testing cut-offs increase sensitivity for non-*albicans Candida* species bloodstream infections

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Abstract

Purpose: Fungal biomarkers support early diagnosis of invasive fungal infections. In this study, we evaluated the impact of a recent update to the manufacturer-recommended cut-off for beta-1,3-D-glucan (BDG) testing (Fujifilm Wako BDG assay) on sensitivity and specificity for the detection of candidemia. Additionally, we compared the performance with tests for *Candida* antigen (Ag by Serion ELISA antigen *Candida*, Virion\Serion) and anti-mannan antibodies (Ab by Hemkit Candida IHA, Ravo Diagnostika).

Methods: Sera of 82 patients with candidemia, which were sampled with a maximum distance of ±14 days from the date of sampling of the corresponding positive blood cultures, were retrospectively analysed for BDG, Ag and Ab. Results of BDG testing were compared with results from sera of 129 patients with candidemia from a different hospital.

Results: Sensitivity of BDG testing (47%) was higher than for Ag (17%) or Ab (20%). By combining Ag and Ab testing, sensitivity was raised to 32%. Lowering the cut-off of BDG from 11 pg/ml to the newly recommended cut-off of 7 pg/ml resulted in a significant increase in sensitivity (47% vs 58%, $p = .01$ and 63% vs 71% $p < .01$). At both centres, the increase was significant in NAC but not in *C. albicans* candidemia. No significant effects on specificity were observed.

Conclusion: BDG testing outperformed Ag and Ab testing and its combination. Lowering the BDG cut-off had no significant impact on specificity. The increase in sensitivity can be mainly attributed to a gain in sensitivity for non-albicans Candida species bloodstream infections.

KEYWORDS
antigen testing, BDG, beta-d-glucan, bloodstream infection, candidemia, mannan

1 | INTRODUCTION

Candidemia is a life-threatening condition with high mortality of up to 60%.[1–3] Particularly patients with malignancies, immunosuppression, abdominal surgery and need for intensive care treatment are at risk.[4] Due to the ongoing development of treatment options in modern medicine, the number of patients at risk is increasing. Therefore, an increasing burden of fungal bloodstream infections was observed over the recent years in many parts of the world.[5–9]

Despite new antifungal drugs and improved treatment algorithms, management of candidemia is challenging and mortality remains high.[9–13] Therefore, early diagnosis is essential, as prompt anti-infective therapy promotes a favourable outcome.[14] Fungal growth from blood cultures (BC) remains the gold standard of

diagnosis of candidemia.[10,13] However, pathogen burden in candidemia is very low, and hence, low sensitivity of about 50% and a rather long time to positivity (compared to bacteremia) are two considerable drawbacks of this technique.[15,16] Thus, testing for biomarkers with a short turn-around time promise to increase diagnostic yield and to speed up diagnosis.

The two *Candida* cell wall polysaccharides beta-1,3-D-glucan (BDG) and mannan (also known as *Candida* antigen, Ag) as well as anti-mannan-antibodies (Ag) are established targets for serological testing. BDG is considered an (almost) panfungal compound of the cell wall of pathogenic fungi—with some exceptions, for example *Blastomyces* spp. and *Mucorales* spp. Several tests for the detection of BDG have been developed. Most performance data are available for the Fungitell test (Associates of Cape Cod). Yet, the number of experience reports focusing on the Wako BDG assay (Fujifilm Wako Pure Chemical Corporation) is increasing. The basis of all BDG assays available on the market is to quantify the set-off of a horseshoe crab coagulation cascade, which is triggered by BDG. This cascade is extremely sensitive, and current BDG assays are capable of detecting very low amounts of the biomarker. In a review on non-cultural diagnostic tests for invasive *Candida* disease, they are more sensitive in detecting candidemia than Ag and Ab assays.[15] At the same time, Gram-negative bacteria which have BDG in the cell walls, such as Agrobacterium spp., and which may cause false positive results near to never occur in humans. One should therefore be cautious and not assume that a general bacteremia may cause false positive results. Cellulosic filters, membranes and gauze employed in medical procedures as well as production-related contaminated medication may contain BDG and may lead to false-positive results.[17] Consequently, BDG assays are less specific for the detection of candidemia compared to Ag and Ab assays. Two tests are currently commercially available for the detection of *Candida* Ag (Platelia Candida Ag-Plus, Bio-Rad] and Serion ELISA antigen *Candida* (Virion\Serion). For the detection of the corresponding anti-mannan antibody (Ab), two commercial tests can be applied: the Platelia Candida Ab-Plus (Bio-Rad) and Hemkit Candida IHA (Ravo).

In this study, we retrospectively evaluated the performances of the Hemkit Candida Ab test, the Serion Ag ELISA and the Wako BDG assay (established and improved cut-off) in the detection of candidemia in a clinical cohort.

2 | METHODS

2.1 | Study population

This study was conducted at the University of Würzburg and at the Ludwig Maximilians University (LMU) Munich. We retrospectively identified sera derived from routine diagnostic of 82 patients with candidemia that were treated in the University Hospital of Würzburg in the period from 2009 to 2018, which were sampled with a maximum distance of ±14 days from the date of sampling a corresponding positive BC (Day 0). Only one episode of candidemia per patient was included in the study. If more than one serum sample was available within the timespan, the sample closest to the corresponding blood culture was analysed. As controls, we included sera of patients ($n = 34$) with either *S. aureus* or *E. coli* bacteremia from the same period. Additionally, we included results of a comparative cohort of patients with candidemia (129) or bacteremia (91) from University Hospital of LMU Munich, which in part has been published previously.[18] The sera of these patients had been tested for BDG at the Max von Pettenkofer Institute at LMU Munich.

2.2 | Microbiological methods

Blood was directly inoculated into BacT/Alert FA (aerobic culture) and FN (anaerobic culture) bottles (Biomérieux, Marcy l'Etoile, France). BCs were incubated at 37°C using the automated Biomérieux BacT/Alert 3D system for 7 days until flagged positive. Species identification was performed via matrix-assisted laser desorption ionisation—time of flight mass spectrometry (MALDI TOF MS, BioMérieux). Time to positivity (TTP) was analysed and was limited to scaling in days.

BDG in serum samples was measured with a turbidimetric BDG assay (Wako BDG assay, Fujifilm Wako Pure Chemical Corporation, Neuss, Germany) with a previously established cut-off of ≥11 pg/ml and a recently newly introduced cut-off of ≥7 pg/ml. The manufacturer introduced the latter in 2021 as the new officially recommended cut-off. Ag was quantified by a *Candida* antigen ELISA (Serion ELISA antigen Candida, Virion\Serion, Würzburg, Germany). Ag levels ≥2.6 U/ml were considered positive, as recommended by the manufacturer. Ab testing was performed by indirect haemagglutination test (IHA, Ravo, Freiburg, Germany). Cut-off titres for positive results are >1:320. The manufacturer's instructions were applied for all tests.

2.3 | Statistical analysis and ethics approval

Data processing and statistical analysis were performed using Microsoft Excel (Microsoft) and GraphPad Prism 5 or the GraphPad McNemar's exact test online calculator (GraphPad Software). The McNemar's exact test was used for comparison of sensitivities and specificities and the Mann–Whitney test for the comparison of medians, both with an α-level of 0.05 assumed to be statistically significant. This retrospective study was reviewed and approved by the ethics committees of the University Hospital of LMU Munich (Ethikkommission bei der Medizinischen Fakultät der LMU München) and of the University Hospital of Würzburg, and a waiver for informed consent was granted.

3 | RESULTS

3.1 | Patients and pathogen characteristics

We identified 82 episodes of candidemia in 82 patients (67% male, median age of 62 years [IQR 52–72]. *C. albicans* ($n = 54$, 66%) was the most frequently observed pathogen. Candidemia by non-*albicans*

Candida (NAC) species (27, 33%) were caused by *C. glabrata* (8), *C. parapsilosis* (7), *C. krusei* (5), *C. tropicalis* (4), *C. guilliermondii* (1), *C. kefyr* (1) and *C. lusitaniae* (1). In one patient, polymicrobial candidemia caused by *C. albicans* and *C. krusei* was detected.

Median time to positivity of the blood cultures was 2 days ranging from 0 to 7 days. Median gap between collection of the serological sample and the blood culture was +1 day, (IQR 0–4 days, range −14 to +11 days.)

With respect to the sample volume, testing for BDG, mannan and the Ab assay was performed for 73 (47 CA, 25 NAC), 65 (41 CA 23 NAC) and 71 cases (48 CA, 22 NAC).

3.2 | Host factors

Serological testing showed no differences related to patient´s sex (BDG 30/49, 61% vs 12/24, 50%, Ag/Ab 14/42, 33% vs 6/21, 29%) or the type of in-hospital treatment—ICU vs non-ICU—(BDG testing: 23/38 (61%) vs 19/35 (54%); Ag/Ab testing: 10/31 (32%) vs 10/32 (31%); Table 1).

3.3 | Sensitivities

The sensitivity of BDG testing (cut-off 11 pg/ml) was the highest of all tests applied (34/73, 47%) with a maximum testing result of 544.9 pg/ml. Ag was detected in 11 of 65 (17%) patients (maximum result 30.9 U/ml). Ab testing (14/71, 20%) demonstrated similar results. By combining Ag and Ab testing, sensitivity was raised to 32% (20/63 Ag OR Ab). In 63 matched samples, BDG was detected in 29 (46%) cases of candidemia compared to 20 (32%) cases by combined testing of Ab OR Ag ($p = .07$). Results are summarised in Table 2 and Figure 1A–C. Receiver operating curves (ROC) of BDG and Ag (Mannan) are illustrated in Figure 2.

BDG testing as well as combined testing for Ab OR Ag was generally more successful in candidemia caused by *C. albicans* (54% and 37%) than by NAC (32% and 23%, Table 1). Compared to BDG testing, the sensitivity for detection of candidemia by *C. albicans* and by NAC was lower in both, Ag (15% vs 11%) and Ab (20% vs 11%) testing.

Lowering the cut-off of BDG from 11 pg/ml to 7 pg/ml resulted in an increased sensitivity (34/73, 47% vs 42/73, 58%, $p = .01$) but led to one additional false-positive result (Table 2). Importantly, this increase can mainly be attributed to the enhanced detection of NAC. In patients with candidemia by NAC 8/25 (32%) vs 14/25 (56%, $p = .04$), episodes were detected by the conventional (11 pg/ml) and the improved cut-off (7 pg/ml). In 45 cases of *C. albicans* candidemia, the reduction of the cut-off only resulted in a non-significant minor increase in the detection rate (26/45, 57% vs 28/45, 62%, $p = .48$).

The time period of serum sampling had an impact on the positivity rate of BDG and Ag testing, but not the positivity rate of Ab testing: in sera sampled ±1 day to the BC sampling date, positive results (BDG 63%, mannan 29%) were more likely than in samples collected with a larger gap to BC (±3 days BDG 53%, mannan 25%, ±7 days, BDG 44%, mannan 18%). For Ab testing, the positivity rate was 23% with no difference in the aforementioned periods.

TABLE 1 Influence of *Candida* species, blood culture time to positivity, intensive care unit admission and sex on sensitivity of BDG and combined antigen/antibody testing

	BDG[a] (cut-off 7 pg/ml, $n = 73$[d])		BDG[a] (cut-off 11 pg/ml, $n = 73$[d])		Ag[b] and/or Ab[c] ($n = 63$[d])	
	Postive (n)/all samples (n)	Sensitivity (%)	Postive (n)/all samples (n)	Sensitivity (%)	Postive (n)/all samples (n)	Sensitivity (%)
Candida species						
C. albicans	27/47	57	26/47	55	15/40	37
Non-*C. albicans*	14/25	56	8/25	32	5/22	23
Time to positivity of the corresponding blood culture						
1 day	11/16	69	8/16	50	4/14	29
≥2 days	31/57	54	26/57	46	16/49	33
Intensive care unit admittance						
Intensive care unit	23/38	61	20/38	53	10/31	32
Non-intensive care unit	19/35	54	14/35	40	10/32	31
Sex						
Male	30/49	61	26/49	53	14/42	33
Female	12/24	50	8/24	33	6/21	29

[a]Beta-1,3-D-Glucan,

[b]candida antigen (mannan),

[c]anti-mannan antibody,

[d]in one patient candidemia with *C. albicans* and *C. krusei* was detected. This case is not included in the analysis of sensitivity of *Candida spp.* but in all other analysis in Table 3.

TABLE 2 Sensitivities and specificities of antigen and antibody tests and their combinations

	Candidemia		Controls	
	Positive samples/all samples	Sensitivity (%)	Positive samples/all samples	Specificity (%)
BDG[a] (cut-off 11 pg/ml)	34/73	47	4/34	88
BDG[a] (cut-off 7 pg/ml)	42/73	58	5/34	85
BDG[a] (cut-off 11 pg/ml) (Comparative cohort)	78/129	60	4/46	91
BDG[a] (cut-off 7 pg/ml) (Comparative cohort)	90/129	70	4/46	91
Ag[b]	11/65	17	0/34	100
Ab[c]	14/71	20	3/34	91
BDG[a] (cut-off 7 pg/ml) and/or Ag[b]	38/65	58	4/34	88
BDG[a] (cut-off 7 pg/ml) and/or Ab[b]	45/67	67	7/34	79
Ag[b] and/or Ab[c]	20/63	32	3/34	91

[a]Beta-1,3-D-Glucan,
[b]candida antigen (mannan),
[c]anti-mannan antibody.

The chronological sequence of serum sampling, before or after the positive BC, did not significantly affect the sensitivity of BDG testing. The positivity rate was 47% for the period from Day −7 to Day 0 and 43% from Day +1 to Day +7. Contrarily, none of the 11 sera obtained before, but 11 of 46 (24%) sampled after Day 0 were positive for mannan (Table 3).

When comparing the performances of the tests in samples of a short (0–1 day) versus a long (2–7 days) time to positivity of the blood cultures indicating candidemia, no significant differences of either BDG or combined Ag/Ab testing were observed.

Our results indicated that the new cut-off significantly increases the sensitivity for NAC candidemia. To confirm this, we re-analysed data obtained in a different university hospital, based on a cohort of 129 patients with candidemia, which in part has been published recently[18] in a retrospective approach (Figure 1D). A gain of sensitivity was observed for the improved vs the conventional cut-off of the BDG Wako assay in patients with candidemia (91/129, 71% vs 79/129, 63%, $p < .01$). Notably, we found a significant increase of sensitivity for NAC candidemia (50/66, 76% vs 42/66, 64%, $p = .01$), but no significant increase for candidemia caused by *C. albicans* (41/63, 65% vs 37/63, 59%, $p = .13$).

3.4 | Specificity

With no false-positive results, the Ag testing was most specific. Three Ab results were false positive, resulting in a specificity 91%.

Lowering the cut-off of BDG from 11 to 7 pg/ml generated one additional false-positive result and a slight, but non-significant decrease in specificity (4/34, specificity 88% vs 5/34, specificity 85%).

Applying the improved cut-off (7 pg/ml) to a comparable control group of patients with bacteremia from the University Hospital of LMU[18] did not result in additional false-positive tests (4/46, specificity 91%).

4 | DISCUSSION

Our study evaluated three different serological biomarkers of fungal infection in serum samples of patients with candidemia.

The distribution of *Candida* species—*C. albicans* being the most prevalent species in this cohort—is concordant with previously reported results.[18,19] Thus, it contrasts other reports solely focusing on epidemiology, demonstrating a strong decrease of the fraction of *C. albicans* in candidemia and a corresponding increase of NAC especially *C. glabrata*, *C. krusei* and *C. parapsilosis* from different centres and regions.[20–22]

In serum samples drawn within short distance to the corresponding positive blood culture, the detection of BDG and Ag was more likely, but the detection rate of Ab was not affected. Only few samples with a time period of more than 7 days from the corresponding blood culture were available, and therefore, significance of sensitivity in this group is limited. In addition, in samples collected prior to more than 7 days from candidemia, it is unclear whether candidemia was already present in these patients at that time.

Insight in the kinetics of BDG, Ag and Ab during candidemia are limited and data mainly derive from case–control studies and non-systematically drawn sera[23–26] The German society for haematology and medical oncology (AGIHO) moderately supports a recommendation for use of BDG screening in patients at risk of

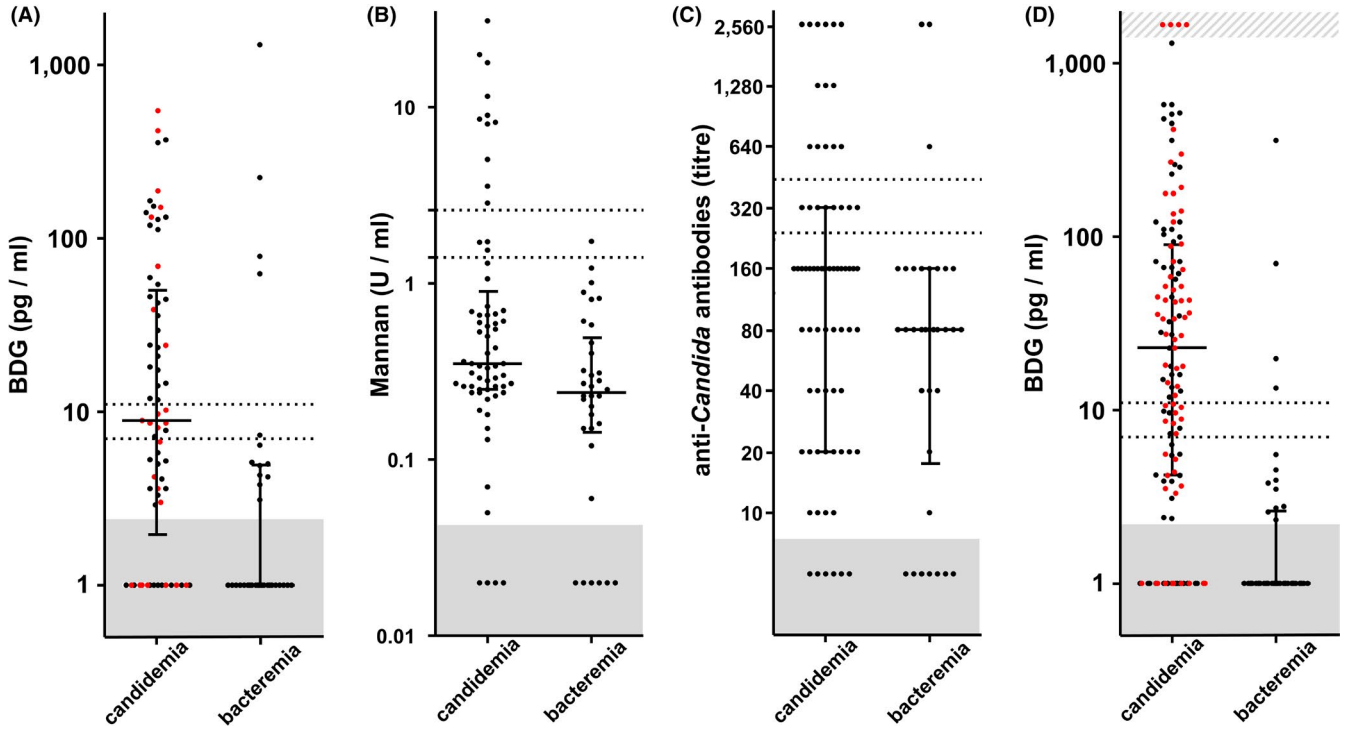

FIGURE 1 Scatter blots of β-1,3-D-glucan (BDG) results obtained in the Würzburg (A) and the Munich (D) study site, of the *Candida* antigen (Mannan) EIA results (B), and the anti-*Candida*-antibody haemagglutination assay (C). Horizontal bars indicate the median and interquartile ranges. Dotted lines indicate the novel and the outdated cut-offs for BDG (A and D), and the upper and lower cut-offs of the borderline measurement range for the antigen EIA and the haemagglutination assay (B and C). Results beneath the limit of detection are plotted in the grey shaded area. For clarity and scale comparability, BDG results > 4000 pg/μl were plotted not to scale but in a grey cross-hatched area (D). For BDG analysis (A, D), measurement results of *C albicans* and NAC candidemia were depicted as black and red dots respectively

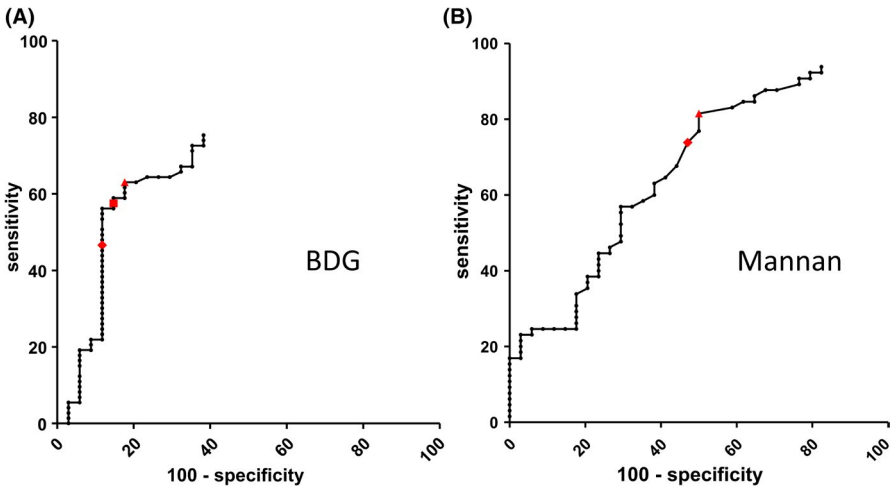

FIGURE 2 Receiver operating curves (ROC) of beta-1,3-D-glucan (BDG) and Mannan. (A) Red square, triangle and diamond indicate the BDG cut-offs of 7.0 pg/μl (novel recommendation by the manufacturer), 5.2 pg/μl (highest Youden's index), and 11 pg/μl (outdated recommendation by the manufacturer). (B) Manufacturer-recommended Mannan EIA cut-off (2.6 U/ml) and the optimal cut-off according to Youden's index are indicated by a red diamond and triangle respectively

fungal infection.[27] Our finding of the time-dependent sensitivity of BDG from the corresponding positive blood culture, emphasises the need to repeat sampling upon clinical suspicion of candidiasis and the pitfall to rely on negative screening results of a weekly routine screening.

The sensitivity of 47% of the Wako BDG assay was higher than testing for Ag, Ab or their combination. This is in good agreement with previously published results.[15,18,28,29] Most of these studies, however, relied on the use of two different assays, that is Fungitell BDG assay and the Platelia system (combination of Platelia Candida Antigen Plus and Antibody Plus, Bio-Rad). In our study, of Ag (Mannan kit, Virion\Serion) and Ab (Hemkit Candida IHA, Ravo) via HAT was as low as 32%. Even upon exclusion of all specimen sampled more than ±3 days from the corresponding BC, sensitivity only increased to 44%. Dichtl et al found a sensitivity of 54% when the same combination of tests in a candidemia cohort was applied. No further data on the performance of the Hemkit assay are available, and only two small-scale studies compared

TABLE 3 Sensitivity of beta-1,3-D-Glucan, candida antigen (mannan), anti-mannan-antibody and combined testing by Δt of the serum sample and the corresponding blood culture growing *Candida* spp

	BDG[a] cut-off 11 pg/ml		BDG[a] cut-off 7 pg/ml		Ag[b]		Ab[c]		Ag[b] and/or Ab[c]	
	Positive samples/all samples (n)	Sensitivity (%)	positive samples/all samples (n)	Sensitivity (%)	Positive samples/all samples (n)	Sensitivity (%)	Positive samples/all samples (n)	Sensitivity (%)	Positive samples/all samples (n)	Sensitivity (%)
all (±14 days)	34/73	47	42/73	58	11/65	17	71	20	20/63	32
±1 day	17/27	63	19/27	70	7/24	29	6/26	23	10/23	43
±3 days	21/40	53	24/40	60	9/36	25	9/39	23	15/34	44
±7 days	28/63	44	36/63	57	10/56	18	14/62	23	19/54	35
−14 to −8 days	2/5	40	2/5	40	0/5	0	0/5	0	0/5	0
−7 to 0 day	8/17	47	10/17	59	0/14	0	3/14	21	2/13	15
+1 to +7 days	20/46	43	26/46	57	10/42	24	11/48	23	17/41	41
+8 to +14 days	4/5	80	4/5	80	1/4	25	0/4	0	1/4	25

[a]Beta-1,3-D-Glucan,
[b]candida antigen (mannan),
[c]anti-mannan antibody.

the performance of the Virion\Serion Mannan Kit.[30,31] Lunel et al. evaluated the Virion\Serion Mannan Kit and the Platelia Candida Antigen Plus in 21 patients undergoing myeloablative chemotherapy with proven invasive *Candida* infection. Sensitivity of the Virion\Serion Mannan Kit was equal to the Platelia Candida Antigen Plus in a cohort of patients with neutropenia <15 days and superior (46% vs 18%) in a cohort of patients with neutropenia >15 days. Debusmann et al. evaluated the assays in a cohort of haemato-oncological and a cohort of abdominal surgery patients (total of 15 patients). In both cohorts, sensitivity of the Virion\Serion Mannan Kit was superior to the Platelia Candida Antigen Plus assays. The generalisability of both studies is limited by their small sample size and their longitudinal approach.

The current ESCMID guideline recommends the combination of Ag and Ab testing in suspected cases of candidemia and states that it could be used to rule out candidemia therefore avoiding unnecessary use of antifungals.[10] However, this recommendation is based on the combination of the Platelia Candida Antigen Plus and Antibody Plus assays, which in combination have been characterised to have a sensitivity of 83%.[32] Considering the low sensitivity of Ag and Ab testing in our study, the ESCMID recommendation to detect or rule out candidemia by the combination of Ag/Ab testing might not be applicable if assays others than the Platelia assays operated.

Two studies recently evaluated the performance of the Wako BDG assay in candidemia: Dichtl et al. reported a sensitivity of 67%, and Friedrich et al., who compared the Fungitell and the Wako BDG assays, reported a sensitivity 43% for the Wako BDG test, which is comparable to our findings.[18,19] This difference might be explained by the nature of the study population defined by the inclusion criterion of BC positivity: at our centre and in the study by Friedrich et al. the blood culture system in operation was the BacT/Alert 3D (bioMérieux), whereas in the study by Dichtl et al. BD BacTec FX blood culture system (Becton, Dickinson and Company) was in use. Interestingly, in a study with BC vials spiked with yeasts, the BacT/Alert system detected growth in 135 of 150 (90%) vials while the BacTec 9240 system detected growth in only 100 of 150 (66%) vials.[33] Furthermore, all *Candida* species spiked into BD BacTec FX that remained undetected were NAC whereas all *C. albicans* spiked vials were positive. We therefore speculate that the low sensitivity of the Wako BDG assay in the study by Friedrich et al. and in our study might be explained by the ability of the BacT/Alert blood culture system to detect candidemia in patients with lower organism burdens and hence lower BDG burdens. Our observation of lower sensitivities of Ag (17% vs 30%) and Ab (20% vs 40%) compared to the study of Dichtl et al. also points in the direction that sensitivities of fungal antigens have to be considered in the light of the applied blood culture system. Under the assumption that lower fungal cell counts are associated with lower antigen production, the hypothesised impairment of the BD BacTec to detect low-level fungal burdens might be mirrored by the high median BDG value of patients with NAC candidemia detected by the BacTec system. This demonstrates the impact of the applied BC system on the positivity rates in detecting candidemia.

The reduction of the manufacturer´s cut-off of 11 pg/ml of the Wako BDG assays has been proposed for the detection of

candidemia, other invasive *Candida* infections and *Pneumocystis jirovecii* pneumonia previously.[19,34,35] Indeed, the manufacturer introduced a new cut-off of 7 pg/ml in 2021. Upon this adjustment, the sensitivity in this study increased from 47% to 59% with only a minor loss of specificity (88%–85%). In the additionally analysed cohort of LMU of 129 patients with candidemia, the sensitivity rose from 63% to 71% with no decrease in specificity. De Carolis et al. reported a considerable increase of sensitivity of the Wako BDG assay for invasive candidiasis from 91% to 99% and a decrease of specificity from 99.5% to 97% by reducing the cut-off to 7 μg/ml.[34] Friedrich et al who compared the Fungitell test to the Wako BDG assay, found an even stronger increase in the sensitivity for candidemia from 43% to 73% upon lowering the cut-off to 3.8 pg/ml. However, this came with a specificity decline from 98% to 91%.[19]

In clinical samples, the reduction of cut-off to 7 pg/ml resulted in a significant overall increase of sensitivity in patients with candidemia. This increase was attributed to a relevant number of sera with a BDG value between 7 and 11 pg/ml and hence a significant increase of sensitivity in patients with candidemia by NAC but not by CA. This was confirmed in an independent cohort of patients in a different hospital. In our cohort, of eight samples, which additionally resulted positive upon reduction of the cut-off, six derived from patients with NAC candidemia. Likewise, in the cohort of Dichtl el al, eight of twelve additionally detected cases had bloodstream infections with NAC. In both studies, this reflects a considerable overrepresentation of NAC among the additionally detected cases. Hence, especially in centres and regions observing an epidemiological trend towards an increased fraction of candidemia by NAC, the reduction of the cut-off could by beneficial.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Johannes Forster: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – original draft (equal); Writing – review & editing (equal). **Karl Dichtl:** Formal analysis (equal); Investigation (equal); Methodology (equal); Resources (equal); Visualization (equal); Writing – review & editing (equal). **Johannes Wagener:** Conceptualization (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – review & editing (equal).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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