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Value of beta-D-glucan and CAGTA biomarkers in diagnosing invasive candidiasis among medical and surgical patients
Lo Cascio, G. *et al.* (2018)

Performance of the association of two markers for invasive candidiasis (IC): (1-3)-beta-D-glucan and Candida albicans germ tube antibodies (CAGTA)
Marchi, E. *et al.* (2018)

Invasive candidiasis (IC) plays an important role as severe infection, constantly rising within hospital wards with intensive and medical-surgical regimen. Diagnosis and treatment are difficult because of the absence of pathognomonic symptoms and due to a low sensitive and time-consuming gold standard (blood culture).

In order to overcome this and to avoid unnecessary and costly empirical antifungal therapies, non-culture-based microbiological assays are proposed as novel early markers of IC.

In these studies, the detection of (1 → 3)-β-d-glucan (BDG) and antibodies against Candida (*C. albicans* germ tube antibodies, CAGTA) are analysed as single tests and in association.

Lo Cascio, G. *et al.* evaluate whether the association of BDG and CAGTA can lead to an earlier and more specific diagnosis of IC in medicine and surgery wards patients and therefore a timely targeted therapy can be initiated.

The performance of the single tests BG and CAGTA in comparison to their combination and the gold standard (blood culture) showed an improvement in IC diagnosis, with a PPV of 65% and a NPV of 100% when BG and CAGTA are combined.

Marchi, E. *et al.* also showed an increase in sensitivity and NPV when the two markers were associated (93% and 91%, respectively). The accuracy of the individual assays augmented up to 84%, when used in association. Moreover, the specificity of the association of the two markers (75%) was lower than that of CAGTA (89%) and higher than that of BDG (70%) taken alone.

Both publications agree that INVASIVE CANDIDIASIS (CAGTA) VIRCLIA® IgG MONOTEST in combination with BDG considerably improves the IC diagnostics showing an enhanced NPV that may help to optimize the therapeutic strategies of antifungal management.

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O0267 Value of beta-D-glucan and CAGTA biomarkers in diagnosing invasive candidiasis among medical and surgical patients

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Background: Invasive Candidiasis (IC) is the most common Invasive Fungal Infection (IFI) among non-neutropenic patients and diagnosis is based on blood cultures, which sensitivity never exceeds 70%. For these reasons, attempts have been made to identify several biomarkers for early diagnosis. Use of two biomarkers (1-3) -β-D-Glucan (BDG) and CAGTA (*Candida albicans* Germ-Tube Antibody) was found useful among ICU patients. The aim of our retrospective study was to evaluate whether the association of BDG and CAGTA and lead to an earlier and more specific diagnosis of IC in medicine and surgery wards patients and therefore initiate a timely targeted therapy.

Materials/methods: We enrolled patients hospitalized in Departments of Medicine, Geriatric and Surgery from the Verona University Hospital. BDG test on serum for suspected IFI were performed and then CAGTA in CLIA using the ThunderBolt® instrument (Vircell microbiologists- Granada Spain) along with the Kit INVASIVE CANDIDIASIS (CAGTA) VIRCLIA® IgG MONOTEST. Results of blood culture performed in the same day (+/- 2 dd) of serum sample was also evaluated.
PPV, NPV, Sensitivity, and Specificity of the single tests and the association of BG and CAGTA were evaluated.

Results: 257 serum samples of 222 patients were examined: 100 with positive blood cultures for Candida and 157 with negative blood cultures. Isolated Candida species were: *C.albicans*, *C.parapsilosis*, *C.tropicalis*, *C.glabrata* e *C.lusitaniae*; among these, 153 were positive to CAGTA and 169 to BDG, 104 was negative to CAGTA and 88 to BDG. PPV was 61%, NPV was 96%, Sensitivity and Specificity was 96% and59% respectively (Tab.1).

Conclusions: The use of the two associated BDG and CAGTA biomarkers leads to an improvement in IC diagnosis, with an increase in PPV. Therefore CAGTA could be used as a second level test. In addition, *Candida albicans* germ tube antibodies, CAGTA, was also positive during positive blood cultures caused by the *C.albicans*, *C.parapsilosis*, *C.tropicalis*, *C.glabrata* e *C.lusitaniae*, which increases its utility in a wide range of candidiasis.

	Sensibility	**Specificity**	**PPV**	**NPV**

CAGTA and BDG/BC	96%	59%	61%	96%
CAGTA or BDG/BC	97%	54%	57%	96%
CAGTA/BC	94%	62%	61%	94%
BDG/BC	94%	54%	57%	93%

Tab.1

Value of beta-D-glucan and CAGTA biomarkers in diagnosing invasive candidiasis among medical and surgical patients.

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O 0267

Introduction:
Invasive Candidiasis (IC) is the most common Invasive Fungal Infection (IFI) among non-neutropenic patients. The IFI represent around 15% of the hospital infections. The main pathogens responsible for these opportunistic infections are in the 70-90% of the cases the yeasts belonging to the genus *Candida spp.*
IC are constantly increasing even among non-neutropenic patients such as those hospitalized in the Departments of Internal Medicine and Geriatrics, of Surgeries, especially in the surgical treatments of the gastro-intestinal tract, and among patients of the Intensive Care Units (UTI) (1,2).
The main risk factors are: the use of chemotherapeutic drugs, basic diseases such as diabetes mellitus, the increasingly advanced invasive medical-surgical procedures, the placement of endovascular devices (central venous catheter), administration of broad-spectrum antibiotics, abdominal surgery, steroid therapy, immunosuppression status, advanced patient age, parenteral nutrition administration, hemodialysis, mechanical ventilation, long-term inpatient hospital (1,2).
Overall mortality rate is around 60% among CI patients, which it's reduced to 40% when administration of specific and selective antifungal therapies is guaranted (3). The availability of new antifungal drugs is not sufficient to decrease mortality, accurate and timely diagnosis of IC is essential for decreasing patient mortality rates, optimizing the antifungal therapy and reducing hospitalization time.
The diagnosis of IC, today, is based on blood culture, but it requires several days of incubation and has been shown to have sensitivities that never exceed 70% (4). For these reasons, attempts have been made to identify several biomarkers for early diagnosis. International guidelines recommend for diagnosis of IC the use of (1-3) -β-D-Glucan (BG), a test with a high negative predictive value (NPV) (5,6), and the association with *Candida albicans* germ-tube antibodies (CAGTA)was found useful among ICU patients (7).

Objective: The aim of our retrospective study was to evaluate whether the association of BG and (CAGTA) can lead to an earlier and more specific diagnosis of IC in medicine and surgery wards patients and therefore we tried to evaluate the ability of the CAGTA VirClia test to identify infections driven by different species of Candida spp.

Materials and Methods:
We enrolled patients at risk of Invasive Fungal Infection (IFI) hospitalized in department of Medicine, Geriatrics and Surgery from the Verona University Hospital in the period between May 2013 and August 2017.
BDG test on serum were performed and then CAGTA in CLIA using the ThunderBolt® instrument (Vircell microbiologists-Granada Spain) along with the Kit INVASIVE CANDIDIASIS (CAGTA) VIRCLIA® IgG MONOTEST. Each patient was tested from a minimum of 1 sample to a maximum of 5 samples of serum. Results of blood culture performed in the same day (+/- 2 dd) of serum sample was also evaluated.
For **CAGTA test** serum samples were performed according to the manufacturer's recommendations (Vircell Microbiologist S.L. , Granada, Spain). Samples were considered positive above the cut-off 1.1. Results with an index between 1.1 and 0.9 were considered doubtful, results below 0.9 were considered negative.
For **BDG test** the Fungitell assay was performed according to the manufacturer's instructions and BDG concentrations were read and analysed with a BioTek ELX808TM Microplate Reader and GEN5 Software (BioTek U.S., VT, USA). The cut-offs for BDG proposed by the manufacturer were as follows: positive, ≥80 pg/mL; indeterminate, ≥60 to ,79 pg/mL; and negative <60 pg/mL. We considered BDG to be positive if the value was ≥80 pg/mL and negative if the value was <80 pg/mL, so that indeterminate results were classified as negative results.
Routine microbiological tests (blood culture, catheter culture and culture of other clinical samples) were also performed according to conventional clinical practice and local guidelines.
PPV, NPV, Sensitivity, and Specificity of the single tests and the association of BG and CAGTA were evaluated.

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Results: A total of 229 patients, for a total of 280 serum samples, between May 2013 and December 2017 were retrospectively included in the study.
On all serum samples, the BDG was negative on 111 and positive on 169 samples, 100 samples had positive blood cultures for *Candida spp* and 180 were negative; the samples with positive CAGTA were 161, while those with CAGTA negative 119. The results of the single test on the samples were reported in TAB.1.
Table 2 shows the behaviour of biomarkers alone and in combination considering IC when blood culture were positive for Candida. The results of the combination of CAGTA and BDG were: VPP 65%, VPN 100%, Sensitivity 100% and the Specificity 62%.
The results of blood culture showed positivity for different Candida: *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* and *C. lusitaniae*.

TAB.1

NUMBER	CAGTA		BC		BDG	
SAMPLES	POS (%)	NEG (%)	POS (%)	NEG (%)	POS (%)	NEG (%)
280	161 (57.5%)	119 (42.5%)	100 (35.7%)	180 (64,3%)	169 (60.3%)	111 (39.6%)

Conclusions: The presence of complicated patients in medical and surgical wards, with significant risk factors for development of invasive mycoses, demands more often clinicians to difficult diagnosis, as Invasive Candidiasis is. Traditional diagnosis based on blood culture has low sensitivities. For these reasons, attempts have been made to identify several biomarkers for early diagnosis. The use of BDG and CAGTA was studied in ICU, with encouraging results. Our study points attention on medical and surgical patients. Our results show how the use of the two associated BDG and CAGTA biomarkers leads to an improvement in IC diagnosis, with excellent NPV and an increase in PPV in respect of the use of the CAGTA or BDG alone. Moreover CAGTA is done with a fully automated analyser, ThunderBolt® EIA-CLIA (VirClia Chemiluminescence, Vircell microbiologists), which can process up to 24 samples simultaneously, giving result after 50 minutes (Fig.1). INVASIVE CANDIDIASI (CAGTA) IgG Kit contains 24 monotest which allows to optimize the analytical sessions and maximize the cost-effectiveness of the kit thanks to no waste of reagents and maximum stability of the reagents.

In our opinion CAGTA could be used as a second level test, after obtaining a positive BDG, to support anti-candida therapy and to exclude false positives from BDG. Our study also confirmed that CAGTA, while looking for *C.albicans* germ tube antibodies, was also positive during positive blood cultures caused by the following species: *C.albicans*, *C.parapsilosis*, *C.tropicalis*, *C.glabrata* e *C.lusitaniae*.

FIG.1

TAB.2

	Sensit.	Specif.	PPV	NPV
CAGTA and BDG/BC	100%	62%	65%	100%
CAGTA/BC	78%	54%	48%	82%
BDG/BC	97%	60%	57%	97%

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P2368 Performance of the association of two markers for invasive candidiasis (IC): (1-3)-beta-D-glucan and Candida albicans germ tube antibodies (CAGTA)

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Background: Invasive candidiasis (IC) plays an important role as severe infection, constantly rising within hospital wards with intensive and medical-surgical regimen. Diagnosis and treatment are difficult because of the absence of pathognomonic signs and symptoms; moreover, microbiological culture-based analysis (gold standard) suffers of low sensitivity and timing of reporting. All this leads to unnecessary, costly, empirical antifungal therapies, burdened by the onset of resistance and side effects. Therefore, non-culture-based microbiological assays have been proposed as novel early markers of IC, such as (1 → 3)-β-d-glucan (BDG) and antibodies against Candida (*C. albicans* germ tube antibodies, CAGTA).

Materials/methods: Retrospective study conducted on 58 sera (rendered anonymous), previously tested for BDG (cut-off 80 pg/ml). We tested the presence of CAGTA, by Vircell kit assay, on 30 sera of patients with documented IC: 27 candidaemie and 3 biopsies positive. Moreover, controls (non-IC) included 28 samples from patients with blood cultures positive for bacteria and 19 negative blood culture sera. The CAGTA assay was performed using several serum dilutions, 1:40, 1:80, 1:160 (as indicated by the manufacturer) and 1:320. The results, in fluorescence, were expressed as arbitrary fluorescence units (AFU) and interpreted using the cut-off value provided by ROC curves (Youden index).

Results: The sensitivity (Se) and negative predictive value (NPV) of CAGTA (52% and 64%, respectively) and of BDG (87% and 84%, respectively) observed when individually tested, greatly increased when the two markers were associated (93% and 91%, respectively). The accuracy of the individual assays augmented from 70% (CAGTA) and 81% (BDG) to 84%, when used in association. Moreover, the specificity of the association of the two markers (75%) was lower than that of CAGTA (89%) and higher than that of BDG (70%) taken alone. Finally, Se, NPV and accuracy of CAGTA consistently increased when sera from C*. glabrata* patients were excluded.

Conclusions: The performance of CAGTA improves excluding *C. glabrata* cases. The performance of both markers increases significantly when used in combination; in particular, the enhanced NPV may help to improve the therapeutic strategies of antifungal *stewardship.*

Performance of the association of two markers of invasive candidiasis: (1→3)-β-D-glucano (BDG) and *Candida albicans* germ tube antibodies (CAGTA)

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INTRODUCTION: Invasive candidiasis (IC) plays an critical role among severe infections, in particular in elder population, immunocompromised individuals, intensive care unit (ICU) patients especially surgical ones.
Diagnosis and treatment are difficult because of the absence of pathognomonic signs and symptoms and limits of culture-based analysis (gold standard). Notoriously, the latter is burdened by low sensitivity and long time-to-report. All this leads to unnecessary, costly, often empirical antifungal therapies, related also to the onset of resistance and side effects.
Recently, non cultural markers have been proposed whit the aim of rapidly achieving early diagnosis of IC. This new markers include research in the serum of (1 → 3)-β-D-glucan (BDG) and antibodies to Candida (*Candida albicans* germ tube antibodies, CAGTA).

METHODS: We retrospectively analyzed the level of CAGTA on serum samples stored at -80°C; the level of BG in the studied samples had previously been assessed in a prospectic study at the AOU-Policlinic of Modena. Briefly, we selected 30 samples from proven IC episodes and other 28 from non-IC controls. The 30 IC samples were from *C. albicans* n = 16, *C. glabrata* n = 8, *C. parapsilosis* n = 1, *C. pelliculosa* n = 1, *C. tropicalis* n = 1, while 3 samples had liver biopsies positive for yeasts (compatible with *Candida* spp.), as established by microscopy. The 28 control samples (non-IC) included sera with positive blood culture (*E. faecium* n = 5, *S. penumoniae* n = 2, *P. aeruginosa* + *A. baumannii* n = 2) and negative blood culture (n = 19). The CAGTA assay was performed at 1:40, 1:80, 1:160 and 1:320 serum diluitions. The results, in fluorescence, were captured as arbitrary fluorescence units (AFU) and interpreted using the cut-off provided by ROC curves (Youden index).

Table 1. Performance of CAGTA and BG

Assessment of	Accuracy % (IC 95%)	Sensitivity % (IC 95%)	Specificity % (IC 95%)
CAGTA	**70** (56,3-81,0)	**52** (38,5-65,3)	**89** (77,3-95,3)
CAGTA (excluding *C. glabrata* samples)	**80** (66,9-88,0)	**68** (54,2-79,3)	**89** (77,3-95,3)
BDG	**81** (68,0-88,7)	**87** (74,8-94,1)	**70** (56,2-81,1)
BDG or CAGTA positive	**84** (71,5-91,8)	**93** (82,3-97,6)	**75** (61,5-85,1)

CAGTA: *C. albicans* germ tube antibodies; BDG: (1 → 3) -> β - D- glucan; IC: confidence interval

RESULTS: The CAGTA demonstrated a sensitivity and specificity of 52% and 89%, respectively, with a ROC-AUC of 0,704. Interestingly, an increase in sensitivity was observed, when excluding the *C. glabrata* episodes (from 52 % to 68 %).
The BG showed a sensitivity of 87% and specificity of 70%, with a ROC-AUC of 0,885.
The association of the two markers, CAGTA+BDG, increased the sensitivity to 93% with an overall accuracy of 84%.

CONCLUSIONS: The performance of CAGTA is greatly increased if cases of IC from *C. glabrata* are excluded. The performance indexes of CAGTA and BDG increase when used in combination.

Fig.1 ROC curve of CAGTA and BDG

Dotted lines: 95%CI
Circle: best cut-off (Youden index)

A: best cut-off for CAGTA = 25,86 AFU
B: best cut-off for BDG = 153,1 pg/ml