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Presence of *Candida* cell wall derived polysaccharides in the sera of intensive care unit patients: relation with candidaemia and *Candida* colonisation

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Abstract

Introduction

Prompt diagnosis of candidaemia and invasive candidosis is crucial to the early initiation of antifungal therapy. The poor sensitivity of blood cultures (BCs) has led to the development of fungal glycan tests as a diagnostic adjunct. We analysed the performance of tests for the detection of circulating β-D-1,3-glucan (BDG) and mannan in the intensive care unit (ICU) setting.

Methods

This retrospective, case–control study included 43 ICU patients with candidaemia and 67 controls, hospitalised on the same ward and assessed weekly for yeast colonisation with simultaneous serum sampling; 340 sera taken before and after positive BCs were available for the cases group and 203 for the controls. BDG and mannan levels were determined using the Fungitell® and Platelia ™ Candida Ag tests, respectively.

Results

BDG was detected early in sera from cases patients but was also present in several sera from controls. Increasing the cut-off from 80 pg/mL to 350 pg/mL and 800 pg/mL resulted in sensitivity/specificity ratios of 0.97/0.31, 0.65/0.74, 0.30/0.86, respectively. Detection of mannan was more specific but lacked sensitivity. No obvious correlation was found between BDG and colonisation, but a trend existed between high colonisation and high BDG. Candidaemia relapses were associated with a rise in BDG and mannan but, in contrast to the transient nature of mannan, BDG persisted up to 7 weeks after positive BCs.

Conclusion

A combination of mannan and BDG tests could be used to guide pre-emptive therapeutic decisions in ICU patients.

Introduction

Invasive candidosis (IC) is one of the leading causes of nosocomial infection and *Candida* species rank fourth among the pathogens involved in bloodstream infections [1]. Despite current progress in research and antifungal therapy, the incidence and attributable mortality of candidaemia remain high [2] due to difficulties in the establishment of an accurate and early diagnosis. In the intensive care unit (ICU), candidaemia has a prevalence of 7/1000 patients, with an attributable mortality of >40% compared to 30% for bacteraemia [3]. Mortality increases from 10% if antifungal therapy is introduced within 12 h of the onset of candidaemia to 35% when treatment is initiated more than 48 h after [4]. These figures are worse in cases of septic shock due to *Candida* species [5]. The challenge is therefore to

manage the delay in initiation of antifungal treatment, especially as 50% of cases of IC are not detected by blood cultures (BCs) and 48 h are generally required for yeast isolation [6]. This low sensitivity of BCs was observed in several large post-mortem studies evaluating the sensitivity of BCs for the diagnosis of deep-seated *Candida* invasion [7] and was shown to range from 28% in cases of single organ candidosis to 58% in cases of disseminated invasive candidosis [8]. Improvement of BC systems has only decreased the delay in yeast isolation for certain species without any improvement in the sensitivity [9]. Thus, relying on BCs or waiting for BC results is not appropriate for managing patients at high-risk of IC.

Considering the need for alternatives to BCs for early diagnosis, the Infectious Diseases Society of America (IDSA) and the European Society of Clinical and Microbiology and Infectious Diseases (ESCMID) have recommended the use of non-culture based methods to help make therapeutic decisions [10,11]. Among the surrogate markers, some *Candida* cell wall derived polysaccharides or oligosaccharides resulting from their catabolism (glycans) can be detected in the sera of patients with candidosis. These consist of mannan, a polymer of mannose representing the polysaccharide moiety of molecules from the outer cell wall layers and β-D,1-3-glucan (BDG), a polymer of glucose making up the fibrils in the middle layers. The combined detection of glycan biomarkers and anti-mannan antibodies was also recommended in the last Surviving Sepsis Campaign, for documentation of the microorganisms involved in septic shock [12]. Numerous studies have evaluated mannan and BDG detection tests for the diagnosis of IC in patients with haematological malignancies and in surgical ICU patients, however information about the value of glucanaemia and mannanaemia monitoring is scarce.

In this study, we looked at ICU patients with candidaemia and control patients from the same ward and with the same high risk factors/predisposing conditions for IC with the aim of analysing BDG and mannan levels during hospitalisation in relation to candidaemia onset or *Candida* colonisation. The primary evaluation measure was an assessment of the two tests to make an early diagnosis of candidaemia. In addition, we analysed how these biomarkers could predict candidaemia relapses or a favourable outcome. Finally, we propose a biomarker-based algorithm designed especially for the management of ICU patients, most of whom are at high-risk of IC.

Materials and methods

Patients

This retrospective, case–control study involved adult patients hospitalised in a 50-bed polyvalent ICU department in a tertiary university teaching hospital. The database of the clinical mycology laboratory was screened to select patients with a positive BC for *Candida* over the period 2005–2010. We focused on patients >18-years-old for whom sera were available at least 1 week before and 1 week after the day of candidaemia. The control group consisted of patients hospitalised on the same ward with *Candida* colonisation but no evidence of IC; five body sites (urine, anal swabs, nasal swabs, throat and tracheal aspirates when patients were intubated) were sampled once a week for the semi-quantitative determination of yeast colonisation. The medical files for these patients were analysed retrospectively using a standardised questionnaire to look for arguments for IC based on the criteria previously used by Mohr et al. [13] and derived from the European Organisation for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [14]. We

also looked for evidence of invasive aspergillosis and infection by *Pneumocystis jirovecii* and excluded patients who had criteria for these two opportunistic fungal infections.

Blood cultures

BCs were performed by drawing 10 mL of blood from either the peripheral vein or arterial catheters into Mycosis ICF vials incubated at 37°C for up to 7 days in a Bactec FX System (Becton Dickinson, Le Pont de Claix, France).

Measurement of BDG in serum

This was performed using the Fungitell® kit (Fungitell®; Associates of Cape Cod Inc., Falmouth, MA, USA), following the manufacturer's instructions. The recommended cut-off value of 80 pg/mL was used to define positivity. Samples with BDG levels >500 pg/mL were diluted and retested.

Measurement of mannan antigen and anti-mannan antibodies in serum

This was performed using the Platelia™ Candida Ag (mannan) and Ab tests (mannan Ab) (Bio-Rad, Marnes la Coquette, France) according to the manufacturer's instructions. The recommended cut-off values for the mannan and mannan Ab tests used between 2005 and 2010 were 0.5 ng/mL and >10 AU, respectively. Samples with mannan >500 pg/mL were diluted and retested.

Intensity of colonisation

This was determined for each date of sampling in the control group. Each sample was incubated on Chromagar® medium under standard conditions. Presumptive identification was confirmed by ad-hoc tests (Bichrolatex, glabrata RTT, API 20C) and the number of colony forming units (cfu) was scored as followed: 1 = <10 cfu; 2 = 10–50 cfu; 3 = >50 cfu; 4 = >50 confluent. Intensity of colonisation was determined for each date of sampling, by dividing the sum score for each colonised site by the number of sites sampled giving a mean *Candida* load (MCL). An overall score of >4 was possible in the case of isolation of several *Candida* species as we added together the colonisation intensity for each species.

For the patients with candidaemia, data on colonisation were derived from a systematic weekly survey of urine, nasal, tracheal and anal colonisation for the isolation of multi-resistant bacteria, where yeasts were also isolated.

Statistical analysis

Quantitative variables are expressed as median values and 95% confidence intervals (CI). Qualitative variables were analysed using Fisher's exact test and quantitative variables using the Wilcoxon test. Tests were two-tailed. Pearson's correlation test was performed when necessary. A significance threshold of 0.05 was retained. All statistical analyses were performed using EpiInfo V3.5.3. Graphics were drawn using Graphpad Prism6.

Ethical statement

All the sera used in this study were sampled from patients followed in Lille university hospital. When no results were available from routine tests, BDG and mannan levels were determined retrospectively on the residual frozen samples. No additional sampling was necessary. As sera were taken from a registered biological collection, according to french law, patients consent was not required. An Institutional Review Board approval was given by the "Comité de Protection des Personnes Nord-Ouest IV", the ethical committee of our institution.

Results

Description of the study population

A total of 117 patients with candidaemia were identified during the study period; 43 (36.8%) of these had all of the criteria for inclusion in the study. Clinical and demographic data for the candidaemia and control groups are shown in Table 1. The groups did not differ in terms of demography, SAPS and risk factors for *Candida* infection, except for intestinal surgery, exclusive parenteral nutrition and extra-renal epuration. A significant difference in mortality was recorded during both the ICU stay and subsequent hospital stay.

Table 1 Major characteristics of patients

Characteristics	**Cases (N = 43)**	**Controls (N = 67)**	***p* (two-tailed)**
Age (years)	61.0 [50.0-72.5]	60.5 [49.5-70.0]	0.76
Sex ratio (M/F)	2.9	2.3	0.67
IGS2	51.5	53.0	0.23
Surgical patient	37.2	20.3	0.07
Abdominal surgery	30.2	10.5	0.01
Other surgery	7.0	10.5	0.7
Deep venous catheter	97.7	96.8	1
Broad spectrum antibiotherapy	100	98.4	1
Corticoids	30.2	39.7	0.41
Vasopressor therapy	65.1	57.8	0.55
Exclusive parenteral nutrition	37.2	4.7	$<5.10^{-5}$
Extra-renal epuration	62.8	29.7	0.001
Mechanical invasive ventilation	100	98.4	1
Duration of mechanical ventilation (days)	31.5 [14.0-47.0]	28.5 [15.5-42.0]	0.5
Neutropenia	7.0	6.4	1
Antifungal therapy	86.0	18.0	$<5.10^{-5}$
Fluconazole	59.5	54.5	
Voriconazole	13.5	0	
Caspofungin	54.1	54.6	
L-AmphB	5.4	9.1	
5fluorocytosine	2.7	0	
Bacteraemia	58.1	27.0	0.002
Distribution of bacteria			
Gram +	28.0	18.8	
Gram -	56.0	56.2	
Mixed G + and G-	16.0	25.0	
ICU duration of hospitalisation	33.5 [23.5-60.0]	29.0 [19.5-46.5]	0.5
ICU mortality	62.8	38.5	0.02
Hospital mortality	65.1	44.6	0.05

Quantitative data are expressed as mean and [QI25-75]. Qualitative data are expressed as percentage.

The median delay between admission to the ICU and appearance of candidaemia was 19 days (range: 10–31). Only one *Candida* species was found in BCs (*C. albicans* 40.5%, *C. parapsilosis* 23.8%, *C. tropicalis* 19%, *C. glabrata* 16.7%), except in one patient who had both *C. albicans* and *C. parapsilosis*. Weekly microbial surveillance of these patients revealed that all were colonised by *Candida* species. For the control patients, weekly multi-site determination of yeast colonisation revealed that all, except two, were colonized with *Candida* species and approximately one-third was colonised with more than one species: two species (15 patients), three species (8 patients) and four species (2 patients). The relative prevalence of the different *Candida* species was similar to that in the candidaemia group (in decreasing order: *C. albicans, C. parapsilosis, C. glabrata, C. tropicalis*).

Glucanaemia and mannanaemia during the ICU stay

Glucanaemia and mannanaemia in sera from patients with candidaemia are shown in Figure 1A and B, respectively, as a function of weeks of hospitalisation before and after positive BCs (D0). Glucanaemia was observed several weeks before positive BC. The median delay between positive BDG and positive BC was 10 days. Glucan level was maximal the week before positive BC. At the date of positive BC, all sera/patients had BDG levels >250 pg/mL. A global decrease in BDG was then observed after week 3, although BDG persisted in some patients for up to 8 weeks.

Figure 1 Kinetics of BDG and mannan in candidemic patients and controls. Values of BDG **(A)** and of mannan **(B)** were measured in 341 sera available from 43 patients with positive blood cultures. Sera were collected as a function of number of weeks of hospitalisation in the ICU with D0 as the day that the first positive blood culture was taken. Values of BDG **(C)** and mannan **(D)** were measured in 203 sera available from 67 control patients hospitalised on the same ward as a function of number of weeks of hospitalisation (CTL group).

These data were analysed in terms of sensitivity, specificity and likelihood ratios in reference to the kinetics of BDG. The results are shown in Table 2 for the whole period of serum collection. To respond to the question “How can glycanaemia predict the onset of IC?” and to respond to the question “How can glycanaemia reveal IC?” we considered the period from D-7 to D + 7; this analysis was also carried out for patients with negative BCs.

Table 2 Sensitivity (Se), specificity (Sp) and likelihood ratio positive and negative (LR+/−) of glucanaemia (BDG), mannanaemia and anti-mannan antibody (mannan Ab) detection tests, as well as a combination of mannan/mannan Ab, during the whole period of serum collection (Week −8/+8), and the critical period for diagnosis D-7/+7

	Se		Sp		LR+		LR-	
	Week −8/+8	D-7/+7	Week −8/+8	D-7/+7	Week −8/+8	D-7/+7	Week −8/+8	D-7/+7
BDG	100	97.1	30.6	30.6	1.4	1.4	0	0.1
Mannan	38.5	32.3	95.8	95.8	9.2	7.7	0.6	0.7
Mannan Ab	58.1	52.9	66.2	66.2	1.7	1.6	0.6	0.7
Mannan or mannan Ab	79.5	58.8	66.2	64.8	2.4	1.7	0.3	0.6

We then analysed how glycanaemia could predict relapse or a favourable outcome by focusing on the 13 patients who had several episodes of candidaemia. Patients were divided into two groups: (i) those with the continuous isolation of yeasts from blood with intervals of <48 h: two BCs for two patients; three BCs for four patients; five BCs for one patient; and nine BCs for one patient; and (ii) patients with well separated candidaemia episodes corresponding to relapses (n = 5). The BDG and mannan Ab profiles of these patients are shown in Figure 2 and their clinical evolution described in the Additional file 1. This panel of patients is representative of the diversity of conditions encountered in the ICU and susceptibility to IC.

Figure 2 Kinetics of BDG, mannan and mannan Ab in five patients with candidaemia relapses. The dates of subsequent *Candida* isolations from blood are indicated by arrows. All patients, except patient 4 with no detectable mannan, had values above the cut-off for all tests, but with different kinetics. BDG and mannan presented different kinetics of circulation in a single patient. The well-known transient nature of circulating mannan was observed and correlated with an inverse evolution of mannan Ab (patients 1, 2, 3, 5). Interestingly a sharp decrease in BDG was also observed over a short period of time (see patients 3, 4, 5).

When analysing the kinetics of BDG, mannan and mannan Ab in Figure 2, all patients (except patient 4 with no detectable mannan) had values above the cut-off for all tests, but with different evolutions. There were positive slopes for BDG and mannan before secondary candidaemia, but different kinetics of circulation in a single patient. The well-known transient circulation of mannan was observed and correlated with the inverse evolution of mannan Ab (patients 1, 2, 3, 5). Interestingly, a sharp decrease in BDG was observed in some patients over a short period of time (see patients 3, 4, 5).

Besides the patients described above, the other 25 patients had no documented clinical or mycological signs of relapse. The duration of the survey ranged from D6 to D102. At the end of the survey, 21 patients still had BDG above the cut-off values and only four had BDG below the cut-off. When considering the evolution of the BDG curve, a “negative slope” was observed for 10 patients including two who were below the cut-off at D9 and D86, and two who became negative at D71 and D74; all other patients still had BDG >150 pg/mL. Two patients had a “positive slope” close to BC isolation (final points at 310 pg/mL and 760 pg/mL at D6 and D10, respectively). The other patients (n = 13) had “stable BDG levels” above the cut-off including levels as high as 2223, 1030 and 1458 pg/mL at D7, D12 and D47, respectively. Median BDG and median duration of the survey were 314 pg/mL and 18 days, respectively.

Patients hospitalised in the ICU with no evidence of Candida infection

The results of glucanaemia and mannanaemia tests for the control group are shown in Figure 1C and D, respectively, according to the duration of hospitalisation (weeks). As early as the first week of hospitalisation, about 50% of the patients presented at least one serum with BDG above the cut-off. This percentage remained stable during the following weeks, up to more than 1 month of hospitalisation. By contrast, only five sera (2%) taken from three different patients had mannanaemia above the cut-off and this was transient for all of them.

For the patients followed for *Candida* colonisation during hospitalisation, glucanaemia was assessed as a function of *Candida* load at the time of serum sampling. There was no correlation between BDG and yeast load in these patients and 17 (8.4%) sera had values over 1000 pg/mL (Additional file 2). As shown in Additional file 3, BDG was significantly associated with a high global *Candida* load. Analysis of the effect of colonisation on mannan did not show any correlation for the very limited number (n = 3) of control patients with mannanaemia.

Impact of variation of the cut-off on the performance of BDG and mannan detection

ROC curves for BDG and mannan are shown in Figure 3 for the D-7/+7 period. For BDG, for a cut-off value of 1600 pg/mL, sensitivity was 0.05 with a specificity of >0.9. For a cut-off of 800 pg/mL, the respective values were 0.30/0.86. The best sensitivity/specificity ratio (0.65/0.74) was obtained for a cut-off of 350 pg/mL. For mannan, the best sensitivity/specificity ratio was 0.36/0.94 for a cut-off of 0.2 ng/mL. From these results it appears that these two tests could be use to complement each other: mannan has an important contribution to specificity for low BDG values while high BDG levels have improved sensitivity and specificity.

Figure 3 ROC curves for BDG (plain line) and mannan (dotted line) for the period D-7/D + 7. AUC [95%CI] for BDG = 0.71 [0.61-0.81] with p < 0.0001; threshold for the best ratio Se/Sp: 353 (0.65/0.74). AUC [95%CI] for mannan = 0.63 [0.52-0.74] with p = 0.009; threshold for the best ratio Se/Sp: 0.2 (0.36-0.94). When considering how the two tests could complement each other, mannan has an important contribution to specificity for low BDG values while higher BDG values have improved sensitivity and specificity.

Discussion

In this study, we evaluated the diagnostic value of glucanaemia in combination with mannanaemia in sera taken sequentially from ICU patients with candidaemia. In parallel, we also assessed the effect of *Candida* colonisation on serum levels of *Candida*-derived polysaccharides in patients from the same ICU department who did not have candidaemia. Altogether, 543 sera taken from 110 patients were analysed. The method of inclusion was intended to mimic clinical practice where clinicians have to quickly identify any ICU patients needing antifungal therapy, including those with negative BCs [6].

Glucanaemia was detected several days, or in some cases weeks, before the isolation of *Candida* species from the blood. In a pilot study evaluating plasma BDG measurement for the diagnosis of deep-seated mycoses and “fungal febrile episodes”, Obayashi *et al.* reported

a sensitivity of 90% for a cut-off of 20 pg/mL [15]. However, subsequent studies gave disparate results from different colorimetric and turbidimetric methods [16] using glucans as standards, in the absence of knowledge regarding the nature of the molecule(s) detected [17]. With the Fungitell test, the multicentre evaluation of Ostrosky *et al*., including 107 cases of proven candidosis, reported a sensitivity/specificity ratio of 0.60/0.92 for a cut-off of 80 pg/mL, although their use of control sera from healthy subjects could have led to an overestimation [18]. Using hospitalised patients from a large hospital study as controls, a similar sensitivity but lower specificity (70%) was reported [19]; this decreased further in another study involving surgical patients [13].

Impressive sensitivity and specificity (around 0.93) for BDG detection for the diagnosis of candidaemia were reported in septic patients with the early stages of fever [20]. This contrasts with the lower performance of this test reported in other studies [19,21], especially low specificity [22]. Indeed, studies published to date report a wide range of sensitivities and specificities for BDG detection [23]. The impact of neutropenia [24] on BDG circulation is probably worth considering. However, in our cohort, an analysis of sensitivity performed on data collected from patients transferred from an haematology ward suggested that the performance of BDG detection was equivalent to that in non-neutropenic patients (data not shown). Altogether, the best sensitivity/specificity ratio (0.65/074) for BDG was found for a cut-off value of 350 pg/mL, which is very close to that proposed by Jaijakul *et al*. for predictive efficiency [25].

This study clearly shows that the decrease in BDG over the course of the disease was much slower than the decrease in mannan. The mechanism involved in the catabolism of β-glucans may involve antibodies participating in their clearance since the existence of human anti-β-glucan antibodies has now been established [26,27], as well as an increase in these antibodies during candidaemia [28]. A second hypothesis for the difference in persistence of BDG and mannan is serum mannosidases that cause natural degradation/turnover of endogenous components; this is not the case for glucans which, like glucuronoxylomannan of *Cryptococcus neoformans*, are resistant to degradation and are purely exogenous in nature [29]. From a clinical point of view, the slow decrease in BDG that we observed may limit its use for managing antifungal treatment, a conclusion that contradicts a previous study which proposed the follow-up of glucanaemia kinetics as a tool to evaluate the efficacy of echinocandin therapy [25]. However, some patients with candidaemia enrolled in our study had a second increase from their baseline BDG, associated with high levels of mannan before secondary BCs, suggesting that monitoring of glucanaemia and mannanaemia in patients receiving antifungal therapy is a useful strategy to identify patients with relapses.

The observation of persistently high BDG levels also raises the question of their impact on modulation of the immune response via their interaction with membrane and soluble PRRs [30].

In the control group, quantitative snap-shot analysis did not reveal a correlation between BDG and yeast burden. However, analysis of cumulative colonisation revealed a trend for an association with BDG >1000 pg/mL (data shown in Additional file 1).

In contrast to BDG, only three control patients had detectable mannan, in agreement with previous conclusions that the detection of mannan with the Platelia test has limited sensitivity but high specificity (11–13). Here, the unusually high frequency of candidaemia episodes involving *C. parapsilosis* may also contribute to low sensitivity observed. In contrast to the

other more pathogenic *Candida* species, the mannan epitope detected by the Platelia test is poorly expressed on *C. parapsilosis* (11). As in a recent study [31], exclusion of *C. parapsilosis* resulted in a moderate gain in sensitivity (from 38% to 45%). Surprisingly, the specificity of BDG detection was also affected by the exclusion of *C. parapsilosis* cases (from 30% to 45%). This is of interest in view of the steady increase in incidence of *C. parapsilosis* candidaemia [32].

The low sensitivity of mannan detection has been attributed to the circulation of high levels of circulating mannan Ab and soluble lectins such as mannan-binding lectin (MBL) forming immune complexes responsible for the rapid clearance of mannan [33-35]. This observation has led to the recommendation for repeated serum sampling and combined mannan and mannan Ab screening to improve the overall sensitivity of mannan-based diagnosis [36]. This recommendation has never been reevaluated, but it is interesting to observe here once again the relation of the respective slopes where a sharp decrease in mannan Ab is often predictive of a mannan peak and *vice versa*. However, mannan gave the higher positive likelihood ratio during the crucial D-7/D + 7 period (7.7 vs. 1.4 for BDG).

This study has several limitations, related to its retrospective character and possible selection bias linked to the availability of sera from candidaemia patients and the constitution of the control group. Nevertheless, this latter group was representative of the high colonisation levels encountered in ICU patients, as reported in studies involving surgical [37] and medical ICU patients who showed an increase in colonisation as a function of the duration of hospitalisation and cumulative exposure to risk factors [38]. This intense colonisation is itself a risk factor for the development of IC. Indeed, application of the criteria of Mohr *et al*. [13] led us to exclude a high proportion of patients from the control cohort, defined as having possible or probable IC. In our opinion, once this selection was made this group represented appropriate controls for ICU daily practice in order to evaluate the performance of biomarkers at discriminating the transition from colonisation to infection, which concerns up to one patient in 10. The differences between the two populations reported in Table 1 probably reflect both the background and the impact of IC [37,38].

From this kinetic analysis, we propose an algorithm (Figure 4) for pre-emptive treatment based on the principle of biological screening of ICU populations, the majority of whom are at high-risk of IC. As BDG detection is a more sensitive early positive test it appears useful for first-line screening. For BDG values >800 pg/mL, specificity values > 90% require no confirmatory test. For a cut-off of <800 pg/mL and >80 pg/mL, determination of mannan is indicated since the high correlation between a combination of these two positive biomarkers and IC could be an indication for pre-emptive treatment. In other cases, regular monitoring of glucanaemia is indicated since specificity increases by considering two or more successive positive results [13,39] and the cost of serological tests is minimal compared to antifungal treatment administered at excess. The colonisation index should be proposed as a last step despite its usefulness due to the limitations of time constraints and cost.

Figure 4 Biomarker-based algorithm proposed for managing pre-emptive therapy in ICU patients at high risk of developing invasive candidosis.

The retrospective application of this sequential prescription of BDG and mannan detection in our cohort would have led to the treatment of 24 patients on the basis of BDG values over 800 pg/mL and five patients with BDG between 80 and 800 pg/mL associated with positive mannanaemia (total 29 = 70% of patients). When considering early positivity, more than 50%

of the patients (22/41) would have been treated before positive BCs. Among the controls, 10 had BDG and four had mannan (14/67 = 15%). These results from the application of our biomarker-based-algorithm for pre-emptive antifungal therapy compare favorably to empiric treatments, which have been shown not to improve the outcome in cases of fluconazole prescription [40], nor of being cost-effective [41]. Similarly, the results compare favourably with probabilist score-based therapy. Indeed the clinical prediction rule developed by Ostrosky-Zeichner *et al.* captured 10.6% of the patients with proven/probable IC, but also 34.1% of patients without IC [42]; the *Candida* Score of Léon C *et al.* exhibited an area under the ROC curve of 0.774, a sensitivity of 77.6 and a specificity of 66.2 [43]. A limitation to this approach is that laboratory procedures often prevent individual testing and despite the fact that only 2–3 h are needed to obtain the results, sera are often treated as a series, once or twice a week. The periodicity of testing should be adapted for institutions with small numbers of samples or the tests should be performed in a reference centre.

The data presented and discussed here suggest that due to the lack of sensitivity of BCs, managing therapeutic decisions using the *Candida* glycan detection test is not unrealistic now that tests are able to detect molecules in the range of pg/mL with high reliability. It is likely that new more sensitive methods for the detection of glycan biomarkers will be developed, establishing a panel of tests that cannot be ignored for patient care, especially in the ICU.

Conclusion

An algorithm for pre-emptive therapy based on a combination of BDG and mannan detection was derived from a kinetic analysis of a large volume of clinical and biological data collected from patients hospitalised in the same ward and who developed candidaemia or not. The follow-up of BDG and mannan kinetics may also predict relapses. This strategy, which could be adapted to the management of the large number of ICU patients with negative *Candida* BCs, should be validated through large prospective studies.

Key messages

• Detection of *Candida* cell wall polysaccharides in serum is a useful adjunct to blood cultures for the diagnosis of invasive candidosis in the intensive care unit.

• The kinetics and duration of circulation differ between BDG and mannan and between infected patients and colonised controls: BDG is an early sensitive biomarker but has low specificity, while mannan appears later, is less sensitive, but has high specificity.

• Increasing the cut-off for BDG and combining BDG detection with mannan detection can help in pre-emptive therapy.

• Mannan and BDG follow-up is not useful for monitoring treatment efficacy, but an increase in these markers is predictive of relapse.

Abbreviations

BCs, blood cultures; BDG, circulating glucans; IC, invasive candidosis; ICU, intensive care unit

Competing interests

DP and BS received research grants from Bio-Rad. All other authors report no potential conflicts.

Authors' contributions

JP collected data, performed the biological tests, performed the statistical analysis and wrote the paper; BS helped with the selection of sera, helped in the interpretation of the results and wrote the paper; SD participated in the experiments and gave critical analysis in the interpretation of the results; KI participated in the experiments and the analysis of the results; NF helped with the selection of sera from the serum bank and with the biological tests; MK helped to collect clinical data; RF advised on the statistical analysis; DM shared his experience in patient management and helped with the statistical analysis and the development of the algorithm; DP coordinated the work, gave critical advice on the interpretation of the results and wrote the manuscript. All authors read and approved the final manuscript.

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Additional files

Additional_file_1 as DOCX

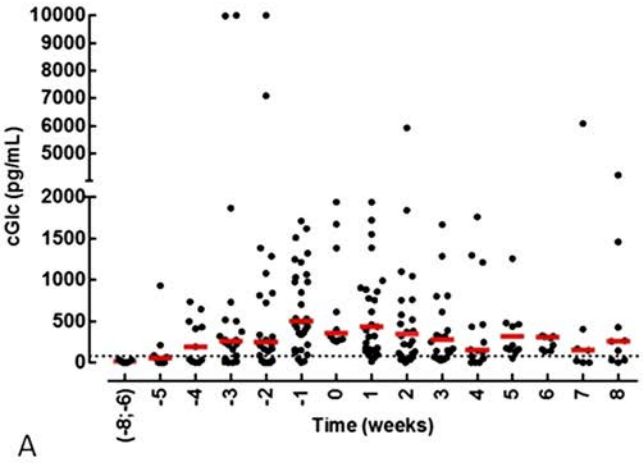
Additional file 1 clinical description of patients with relapses of candidaemia, and for whom kinetics profiles of biomarkers are shown on Figure 2.

Additional_file_2 as TIFF

Additional file 2 graphical representation of the distribution of the glucanaemia reported to the daily fungal load, to show that there is no correlation between colonization and glucanaemia.

Additional_file_3 as TIFF

Additional file 3 graphical representation of the relation between mean global *Candida* load and glucanaemia. This representation shows a trend for an association between a cumulative high level of colonization during all the ICU stay and a high glucanaemia level.



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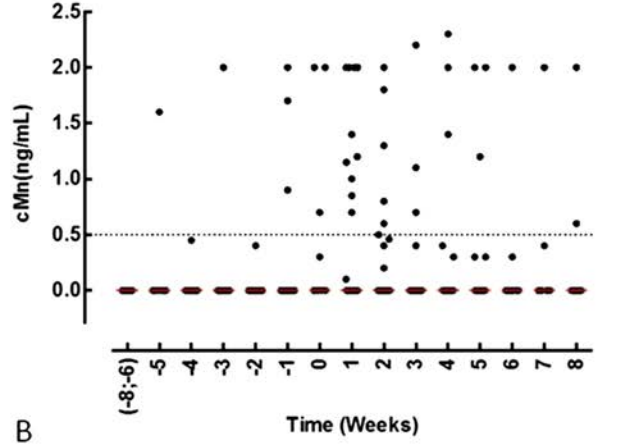

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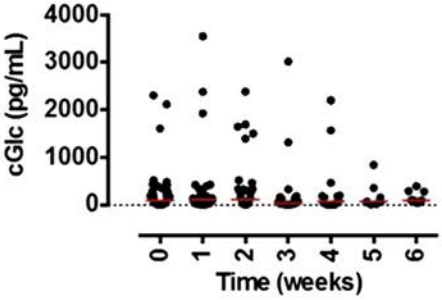

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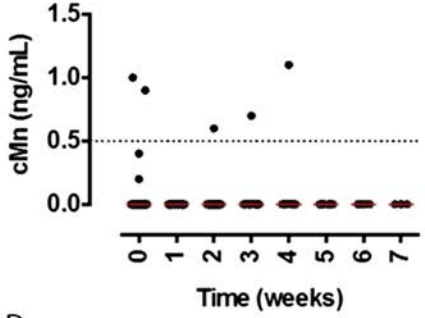


D

Figure 1

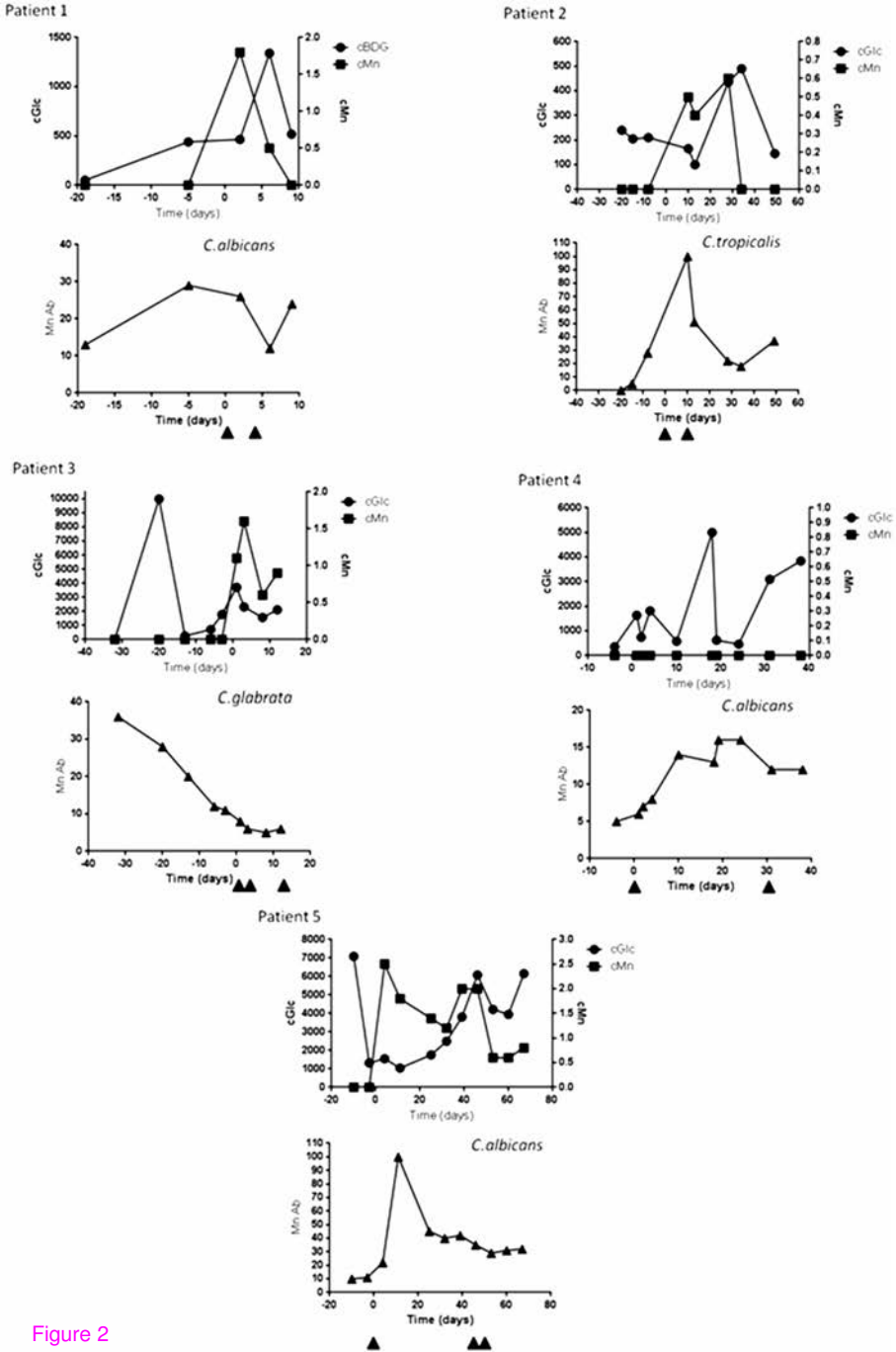
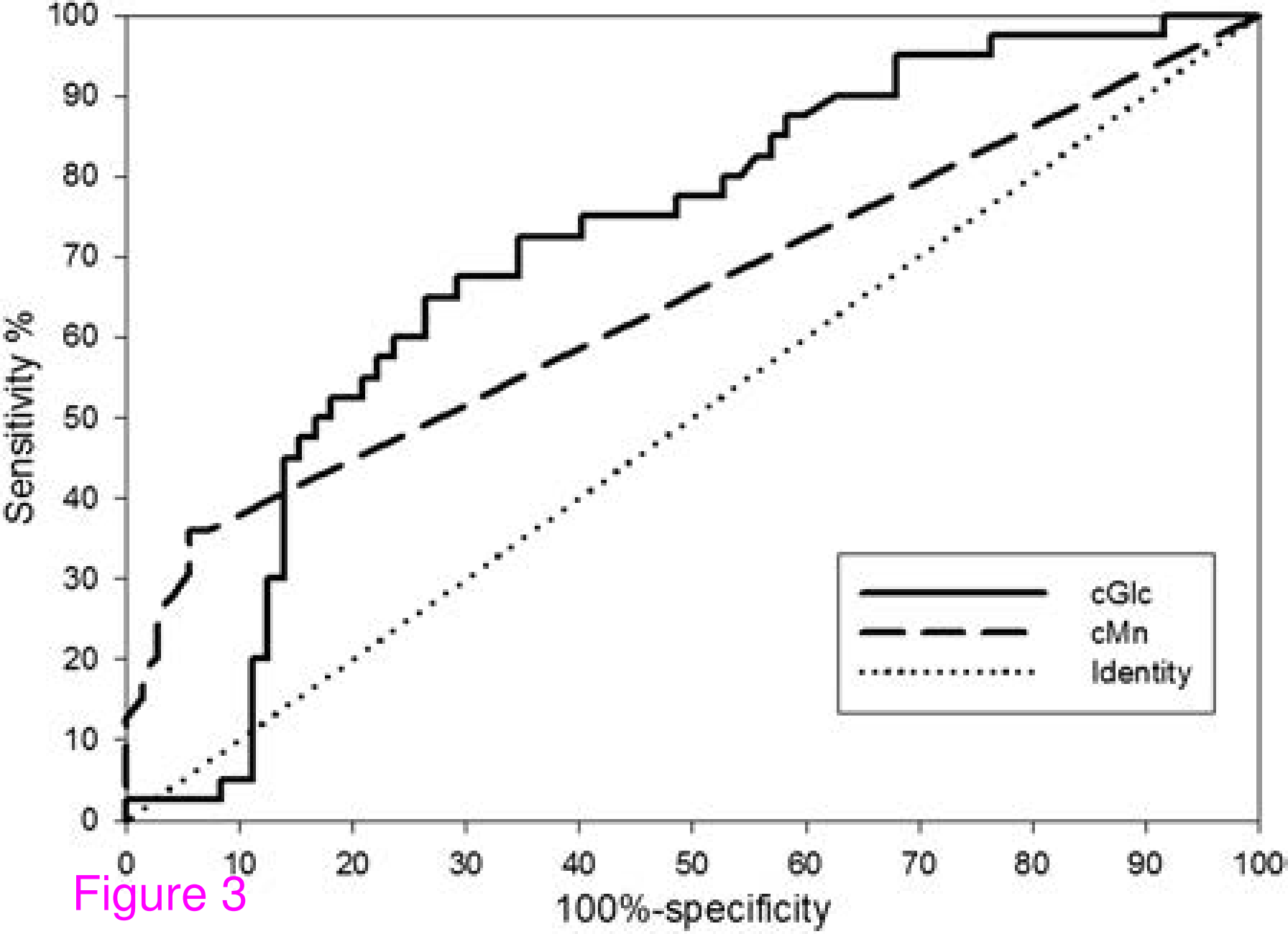

Patient 1
cGlc
cBDG
cMn
Time (days)
C.albicans
Mn Ab
Time (days)
Patient 2
cGlc
cMn
Time (days)
C.tropicalis
Mn Ab
Time (days)
Patient 3
cGlc
cMn
Time (days)
C.glabrata
Mn Ab
Time (days)
Patient 4
cGlc
cMn
Time (days)
C.albicans
Mn Ab
Time (days)
Patient 5
cGlc
cMn
Time (days)
C.albicans
Mn Ab
Time (days)

Figure 2


Sensitivity %
100
90
80
70
60
50
40
30
20
10
0
0
10
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30
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50
60
70
80
90
100
100%-specificity
cGlc
cMn
Identity

Figure 3

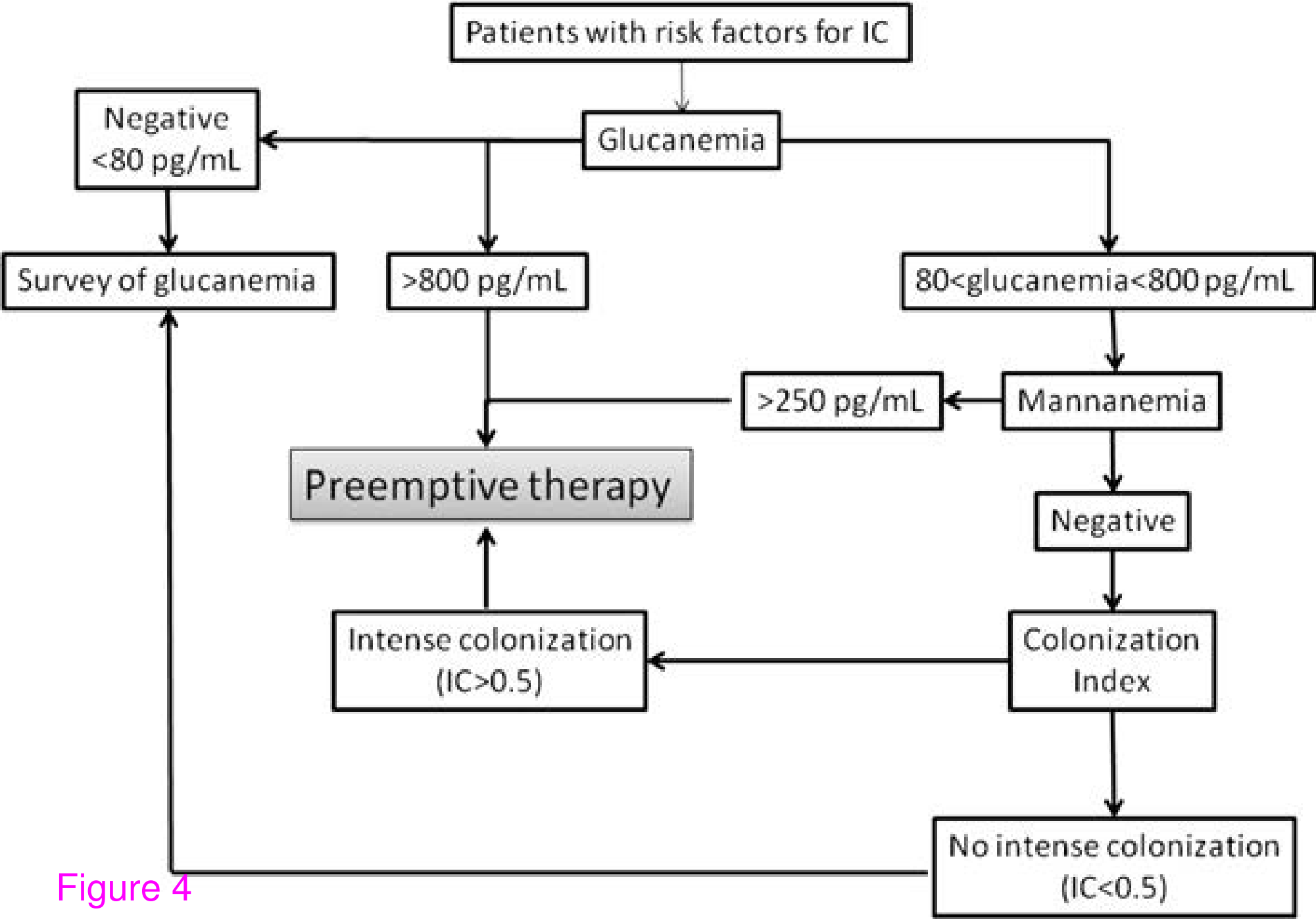
Patients with risk factors for IC
Glucanemia
Negative
<80 pg/mL
Survey of glucanemia
>800 pg/mL
80<glucanemia<800 pg/mL
>250 pg/mL
Mannanemia
Preemptive therapy
Negative
Intense colonization
(IC>0.5)
Colonization
Index
No intense colonization
(IC<0.5)

Figure 4

Additional files provided with this submission:

Additional file 1: 1137021350118496_add1.docx, 12K
http://ccforum.com/imedia/3338927131327444/supp1.docx
Additional file 2: 1137021350118496_add2.tiff, 153K
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Additional file 3: 1137021350118496_add3.tiff, 124K
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