Serum 1,3-β-D-Glucan assay in the diagnosis of invasive fungal disease in neonates

Cheryl Anne Mackay,1,2 Daynia Elizabeth Ballot,1,2 Olga Perovic1,3
1University of the Witwatersrand, Johannesburg;
2Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg;
3Microbiology External Quality Assessment Reference Unit, National Health Laboratory Service, Johannesburg, South Africa

Abstract

Invasive fungal disease is a significant cause of morbidity and mortality in the neonate. The current study aims to assess the 1, 3-β-D-Glucan (BG) assay in a prospective analysis in neonates with suspected fungaemia. A multicentre, prospective cohort study was conducted in Johannesburg, South Africa. The study included 72 neonates with clinically suspected late onset sepsis who were at high risk of fungaemia. A BG assay was performed on each patient and correlated with a sepsis classification based on the full blood count, C-reactive protein and blood culture results as no fungaemia, possible fungaemia, probable fungaemia or definite fungaemia. Sensitivity and specificity of the BG assay at levels of 60 pg/mL are 73.2% and 71.0% respectively and at levels of 80 pg/mL are 70.7% and 77.4% respectively. Positive and negative predictive values at 60 pg/mL are 76.9% and 66.7% respectively and at 80 pg/mL are 80.6% and 66.7% respectively. The area under the receiver operating curve is 0.753. The BG assay is a useful adjunct to the diagnosis of invasive fungal disease in neonates. It does, however, need to be considered in the context of the clinical picture and supplementary laboratory investigations.

Introduction

Neonatal mortality contributes approximately 36% to under-5 deaths globally.1 In South Africa, the neonatal mortality rate is 21/1000 live births with 21% of these deaths being due to severe infections.2 The impact of neonatal sepsis is clearly a significant one. Fungaemia is becoming increasingly important as a cause and is associated with substantial morbidity and mortality in the preterm infant.3,4

Candida species rapidly colonize the skin and mucous membranes of critically ill neonates which can progress to invasive infection. This is particularly so in critically ill neonates who are at increased risk of fungal infection due to immature immune systems, increased permeability of skin and mucous membranes, parenteral nutrition, broad-spectrum antibiotics, central venous catheters, postnatal steroids and mechanical ventilation.4 This high-risk population could benefit greatly from early diagnosis. The diagnosis of sepsis in neonates, including invasive fungal disease, is difficult as the clinical presentation is often subtle and signs are often nonspecific.5 There is no single diagnostic test available to reliably confirm or refute sepsis on presentation. Currently diagnosis is based on a combination of clinical features and laboratory investigations such as the full blood count (FBC), C reactive protein (CRP), erythrocyte sedimentation rate (ESR) and procalcitonin (PCT), amongst others.5,6 Definitive diagnosis depends on a positive blood culture in keeping with clinical features and other laboratory investigations. Blood cultures are often negative in neonates even in the presence of sepsis.7 This is even more problematic with fungal infections as blood cultures are only positive in approximately 50% of cases of invasive candidiasis and less than 10% of invasive aspergillosis.8,9

1, 3-β-D-Glucan (BG) is a component of the outer wall of a number of fungi including Candida species, Aspergillus species and Pneumocystis jiroveci. The antigen is released into the bloodstream during invasive infection due to such fungi and can be detected by the Fungitell™ assay.10 This assay uses enzymes from the Limulus polyphemus amoebocyte lysate and removes bacterial endotoxin-sensitive factor C from the lysate to form a reagent. BG from patient serum binds to Factor G in the lysate and removes bacterial endotoxin-sensitiv factor C from the lysate to form a reagent. BG from patient serum binds to Factor G in the reagent and activates the horseshoe crab coagulation cascade allowing quantitative assay. Values >80 pg/mL are considered positive, 60-80 pg/mL are considered equivocal (repeat testing recommended) and values less than 60 pg/mL are considered negative.10 The sensitivity and specificity of the BG assay for invasive fungal infections is reported as 69.9% and 87.1% respectively.10 Studies to date are based on adult haematological, immunocompromised, cancer and surgical patients. Little information is available with regard to neonates. New diagnostic markers are needed to improve the early diagnosis of fungaemia in newborns. The current study comprised a prospective analysis to determine the usefulness of the BG assay for detection of fungal antigen in clinically suspected neonatal invasive fungal disease at three academic hospitals in Johannesburg.

Objectives

To determine the 1, 3-β-D-Glucan assay in a prospective diagnostic analysis in neonates with suspected fungaemia. To correlate possible, probable or definite fungaemia with BG assay results and determine the sensitivity and specificity of the test in neonates.

Materials and Methods

Study design

A multicentre, prospective cohort study was conducted including 72 neonates admitted to the neonatal units at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Rahima Moosa Mother and Child Hospital and Chris Hani Baragwanath Hospital in Johannesburg.
Patient selection
Patients with clinically suspected late onset sepsis, defined as onset of sepsis after 72 hours of life, were identified and classified as no or high risk for invasive fungal disease. Patients were regarded as high risk for invasive fungal disease and were eligible for inclusion in the study if they had 3 or more of the following criteria: i) Low birth weight (<2500 g); ii) Duration of hospitalisation > 3 weeks; iii) Prolonged invasive (interrupted positive pressure) ventilation or non-invasive (continuous positive pressure) ventilation (>1 week); iv) Systemic antibiotic exposure >72 hours, including poor clinical response to first or second line antibiotic therapy; v) Postoperative patients or patients with abdominal wall defects; vi) Central venous or arterial catheterisation >72 hours; vii) Received total parenteral nutrition (TPN); viii) Splenomegaly; ix) Persistent severe thrombocytopenia (platelet count <100,000/mm² despite second line antibiotic treatment). Patients on systemic antifungal therapy prior to investigation for new onset sepsis were excluded. Patients fulfilling the above criteria were investigated for sepsis as per unit protocol. This included blood investigations (FBC with platelet count, CRP and blood culture) as well as urine cultures, lumbar puncture and radiological investigations where indicated.

Laboratory investigations
The blood investigations included an FBC with platelet count, CRP and blood culture collected in a FAN blood culture bottle (as per laboratory standard operative procedures). In addition to these investigations, a sample of blood was drawn for a 1, 3-ß-D-Glucan assay. The assay was performed using the Fungitell™ assay kit (Fungitell® Cape Cod) and was performed in the Microbiology Laboratory at CMJAH according to manufacturer instructions. The assay was performed in duplicate on each sample and the average of the results obtained was determined. In the case of extremely discrepant results, the results have been radiological investigations were performed at the discretion of the attending physician. Informed consent was obtained from the primary caregiver before inclusion in the study. This study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (approval number M090211).

Diagnostic criteria for invasive fungal disease
Based on the above investigations patients were categorised as one of the following: i) no fungaemia: a high index of suspicion for fungaemia including 3 or more risk factors as described above; blood culture negative; white cell count (WCC), platelet count (<100 000/mm³) and C-reactive protein (CRP) within normal limits; no evidence of colonisation with a fungal organism; OR definite bacterial sepsis; ii) possible fungaemia: a high index of suspicion for fungaemia including 3 or more risk factors as described above; blood cultures negative; no evidence of colonisation; two or more of the following present: abnormal WCC, low platelet count or elevated CRP (>10); iii) probable fungaemia: a high index of suspicion for fungaemia including 3 or more risk factors as described above PLUS evidence of colonisation with a fungal organism in the form of positive stool or non-sterile urine sample (collected by urine bag) for yeasts; blood cultures negative; and one or more of the following are present: abnormal WCC, low platelet count (<100 000/mm³) or elevated CRP (>10); iv) definite fungaemia: positive culture for a fungal organism from a normally sterile site (including blood or tissue culture). The BG results were correlated with the classification as described above, no fungaemia, possible fungaemia, probable fungaemia or definite fungaemia.

Statistical analysis
The sample comprised 72 neonates. Continuous variables (including birth weight and gestational age) were not normally distributed and have therefore been described as median and range. Categorical variables have been described using proportions. Sensitivity and specificity of the 1,3-ß-D-Glucan assay have been determined and the positive and negative predictive values of the test have been calculated. The categories possible, probable and definite fungaemia were combined and considered diagnostic for fungaemia for the purpose of statistical analysis. The category no infection or confirmed bacterial infection were analysed as no fungaemia. Receiver operating curves have been used to further analyse the results.

Table 1. Sample characteristics (n=72).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>45 (62.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>27 (37.5%)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1340 (720-4600)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>31 (26-40)</td>
</tr>
<tr>
<td>Surgical intervention</td>
<td>34 (47.2%)</td>
</tr>
<tr>
<td>IPPV</td>
<td>61 (84.7%)</td>
</tr>
<tr>
<td>CPAP</td>
<td>5 (6.9%)</td>
</tr>
<tr>
<td>HFOV</td>
<td>3 (4.2%)</td>
</tr>
<tr>
<td>TPN</td>
<td>49 (68.1%)</td>
</tr>
<tr>
<td>Central venous/arterial catheter</td>
<td>30 (41.7%)</td>
</tr>
<tr>
<td>Previous antibiotic exposure</td>
<td>72 (100%)</td>
</tr>
<tr>
<td>Empiric antifungal therapy</td>
<td>18 (25%)</td>
</tr>
</tbody>
</table>

Table 2. Correlation of sepsis category with blood culture results and 1,3-ß-D-Glucan assay level (n=72).

<table>
<thead>
<tr>
<th>Sepsis category</th>
<th>n</th>
<th>Positive fungaemia</th>
<th>Positive bacterial culture</th>
<th>Evidence of colonisation</th>
<th>1,3-ß-D-Glucan assay level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>31</td>
<td>0</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

1Sepsis categories as described under methods; Positive culture from a normally sterile site (including blood or tissue culture); 2Blood or non-sterile urine sample (urine bag specimen) positive for yeasts.

Results
The initial sample included 79 neonates of which 72 formed part of the analysis. Of the seven potential candidates removed from the study, two caregivers refused consent, two samples were rejected at laboratory level (one was contaminated and the other was haemolysed), two samples had a laboratory error and one result was uninterpretable. The sample characteristics are summarized in Table 1.

Correlation of sepsis category with blood culture results, evidence of colonisation and 1,3-ß-D-Glucan assay level are presented in Table 2. Fungal culture from a normally sterile site was positive in 10 cases (nine blood samples and one tissue sample). Four of the samples cultured Candida albicans, four cultured Candida parapsilosis, one cultured Saccharomyces cerevisiae and one blood culture was reported as yeast but not identified. The lowest BG assay in cases with a positive fungal culture was 109 pg/mL with seven fungal culture positive cases having a BG assay >500 pg/mL. In the categories possible fungaemia, probable fungaemia, probable fungaemia or definite fungaemia.

Table 2. Correlation of sepsis category with blood culture results and 1,3-ß-D-Glucan assay level (n=72).

<table>
<thead>
<tr>
<th>Sepsis category</th>
<th>n</th>
<th>Positive fungaemia</th>
<th>Positive bacterial culture</th>
<th>Evidence of colonisation</th>
<th>1,3-ß-D-Glucan assay level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>31</td>
<td>0</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

1Sepsis categories as described under methods; Positive culture from a normally sterile site (including blood or tissue culture); Blood or non-sterile urine sample (urine bag specimen) positive for yeasts.
gaemia and probable fungaemia, in which cultu-
res from a normally sterile site remained
negative and invasive fungal disease would
previously not have been diagnosed, there
were an additional 19 BG assay levels
>80 pg/mL.

The sensitivity and specificity of the BG
assay at levels of 60 pg/mL are 73.2% and 71.0%
respectively and at levels of 80 pg/mL are 70.7%
and 77.4% respectively. The positive and nega-
tive predictive values at 60 pg/mL are 76.9%
and 66.7% respectively and at 80 pg/mL are
80.6% and 66.7% respectively. In addition, the
area under the ROC curve is 0.753. Sensitivity,
specificity and positive and negative predictive
values calculated according to varying BG
assay levels are presented in Table 3. ROC sta-
tistics and curve are presented in Figure 1.

Discussion

The current study shows the 1,3-βD-Glucan
assay to be a useful adjunct in the diagnosis of
fungaemia in neonates. Existing recommenda-
tions for positivity and negativity of the assay
appear to be appropriate for the neonatal peri-
dod. Despite reasonable sensitivity and speci-
ficity of the 1,3-βD-Glucan assay, there remain
a significant number of false positive and false
negative results.

Potential causes of false positive reactivity
include patients or specimens exposed to BG
containing products (for example gauzes),
patients undergoing haemodialysis with cellu-
lose containing membranes, patients receiving
intravenous immunoglobulin therapy and
exposure to the antibiotic amoxicillin-clavulu-
lanic acid.11 Albumin, clotting factors and plas-
ma protein concentrates are manufactured
using filters containing high concentrations of
BG and may lead to false positivity. In addition,
the bacterial organisms*Streptococcus pneumoniae
and Alcaligenes faecalis* are known to con-
tain β-1,3-glucan in their cell walls and may
do also cause false reactivity.11 Other bacteria,
specifically gram positive organisms and
*Pseudomonas aeruginosa*, may also cause false
positive reactivity.12,13

The early diagnosis and treatment of fun-
gaemia, which has been shown to decrease
mortality, is challenging.14 The blood culture as
the gold standard of diagnosis is limited by
delays of 18 to 72 hours to achieve positivity.8
In addition, only 50% of invasive candidiasis
and less than 10% of invasive aspergillosis are
blood culture positive. This curtails the poten-
tial survival benefit of earlier antifungal treat-
ment. A reasonable clinical approach to the
neonate with suspected fungaemia is to com-
cence antifungal therapy based on a positive
1,3-βD-Glucan assay result (which can be
obtained within 24 hours) whilst blood culture
results are pending. In the case of a positive
1,3-βD-Glucan assay result and a negative
blood culture result one needs to consider both
the degree of positivity (for example, 1,3-βD-
Glucan assay levels > 250 pg/mL have a speci-
vitie of 96.8%) and the presence of factors
causing false positive reactivity. Results also
need to be interpreted taking the infant’s clin-
cal presentation into consideration.

One of the difficulties in the current study
was the criteria used for diagnosing fun-
gaemia. Use of the blood culture as gold stan-
dard would be ideal but the yield of positive
blood cultures in neonatal sepsis is known to
be low.7 This led to the use of alternative crite-
reria for the diagnosis of fungaemia which,
although unavoidable, may lead to inaccura-
cies in the analysis. A similar dilemma sur-
rounding diagnostic criteria is demonstrated
in a previous study by Odabasi et al.15 In this
study sensitivity and specificity of the BG
assay at a level of 60 pg/mL are reported as
100% and 90% respectively for proven or prob-
able fungaemia and 70% and 96% respectively
for proven, probable or possible fungaemia.

Limitations of the BG assay include the cost
of the test (particularly important in a develop-
ing country such as South Africa) and the lack
of species identification. A positive BG assay
result indicates the presence of one of a num-
ber of possible fungi, including amongst others
Candida species, the most common fungal iso-
late in neonates.3 It is, however, dependent
upon the blood culture for identification and
sensitivity of the causative organism. For this
reason the BG assay may be of particular use
in an epidemic or nosocomial outbreak setting.

Conclusions

The BG assay is a useful adjunct to the diag-
osis of fungaemia in neonates with a predic-
tive value of 0.753 on ROC analysis. BG varies
in specificity, depending on the cut-off values
used, due to a number of false positive results.
The assay therefore needs to be considered in
the context of the clinical picture and supple-
mentary laboratory investigations. Ongoing
research is required into further causes of
false positive reactivity and the change in
assay levels in response to treatment.

References

1. UNICEF. The State of the World’s Children
2008: Child Survival. 2008; Available from:
2. WHO. World Health Statistics 2006. 2006;
Available from: http://www.who.int/who-
A 10-year prospective surveillance of noso-
comial infections in neonatal intensive
care units. Am J Infect Control 2007;35:

---

Table 3. Sensitivity, specificity and positive and negative predictive values at varying 1,3-
βD-Glucan assay levels (n=72)

<table>
<thead>
<tr>
<th>Positive if 1,3-βD-Glucan greater than or equal to</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV1</th>
<th>NPV2</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>0.732</td>
<td>0.710</td>
<td>0.769</td>
<td>0.667</td>
</tr>
<tr>
<td>80</td>
<td>0.707</td>
<td>0.774</td>
<td>0.806</td>
<td>0.667</td>
</tr>
<tr>
<td>150</td>
<td>0.535</td>
<td>0.839</td>
<td>0.828</td>
<td>0.605</td>
</tr>
<tr>
<td>200</td>
<td>0.512</td>
<td>0.903</td>
<td>0.875</td>
<td>0.583</td>
</tr>
<tr>
<td>500</td>
<td>0.317</td>
<td>0.968</td>
<td>0.923</td>
<td>0.517</td>
</tr>
</tbody>
</table>

Figure 1. Receiver operating characteristic curve for the 1,3-
βD-Glucan assay.
183-9.