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Jérémie F. Cohen, Antoine Ouziel, Soraya Matczak, Josephine Brice, Rene Spijker, et al.. Diagnostic accuracy of serum (1,3)-beta-d-glucan for neonatal invasive candidiasis: systematic review and meta-analysis: (1,3)-Beta-D-Glucan to detect neonatal invasive candidiasis. Clinical Microbiology and Infection, 2019, Epub ahead of print. 10.1016/j.cmi.2019.09.010 . inserm-02319288

HAL Id: inserm-02319288

https://inserm.hal.science/inserm-02319288

Submitted on 17 Oct 2019

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Diagnostic accuracy of serum (1,3)-Beta-D-Glucan for neonatal invasive candidiasis: Systematic review and meta-analysis

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Intended category: Systematic Review
Word count: 2952/3500 **References:** 41 **Tables**: 4 **Figures**: 3

ABSTRACT (300/300)

Background: Neonatal invasive candidiasis (NIC) is a leading cause of infection-related morbidity and mortality in preterm neonates. Several studies have shown that (1,3)-Beta-D-Glucan (BDG) was accurate in detecting invasive fungal infection in adults, but studies in neonates are scarce.

Objectives: To obtain summary estimates of the accuracy of BDG detection in serum for the diagnosis of NIC.

Data sources: We searched Medline, Embase, Clinicaltrials.gov, and Google Scholar (inception to July 2019). We checked the reference lists of included studies, clinical guidelines, and review articles.

Study eligibility criteria: We included studies that assessed the accuracy of BDG against a reference standard that defined groups of patients with ordinal levels of NIC probability (e.g., proven, probable, possible) and included fungal blood culture.

Participants: Neonates suspected of having NIC.

Interventions: BDG measurement in serum (Fungitell® assay).

Methods: We assessed risk of bias and applicability using QUADAS-2. We used bivariate meta-analysis to produce summary estimates of diagnostic accuracy at prespecified positivity thresholds of 80 and 120 pg/ml. This study was registered with PROSPERO (CRD42018089545).

Results: We included eight studies (465 participants). Of these, two were judged at low overall risk of bias. There was substantial variability across studies in the reference standards used. At a positivity threshold of 80 pg/ml, summary estimates of sensitivity and specificity of BDG were 89% (95% CI: 80% - 94%) and 60% (53% - 66%), respectively; summary sensitivity for detecting proven cases of NIC was 99% (93% - 100%). At a positivity threshold of 120 pg/ml, summary estimates of sensitivity and specificity were 81% (71% - 88%) and 80% (67% - 88%), respectively.

Conclusions: Because of high sensitivity, BDG seems promising to rule-out NIC. It might be too early to recommend its use because of the scarcity of reliable clinical data, heterogeneity in case definitions, and unstable accuracy estimates.

Abbreviations

BDG: (1,3)-Beta-D-Glucan

CRP: C-reactive protein

NIC: Neonatal invasive candidiasis

IQR: Interquartile range

EORTC/MSG: European Organization for Research and Treatment of Cancer/Mycoses Study Group

95CI: 95% confidence interval

BW: Birth weight

ROC: Receiver operating characteristic

WG: Weeks of gestation

Keywords: Neonatal invasive candidiasis; (1,3)-beta-D-glucan; diagnostic tests; sensitivity; specificity; neonates; systematic review; meta-analysis.

Running title: (1,3)-Beta-D-Glucan to detect neonatal invasive candidiasis

Previous presentation: Preliminary results of this work were presented in part at the 36th Annual Meeting of the European Society for Paediatric Infectious Diseases (ESPID), Malmö, Sweden, May 2018 (E-poster ESP18-0223).

INTRODUCTION

Neonatal invasive candidiasis (NIC) is one of the leading causes of sepsis in neonatal intensive care units. *Candida* species are the third most common infectious agents isolated in late-onset neonatal sepsis (1), with *C. albicans* and *C. parapsilosis* accounting for 80 to 90% of neonatal fungal infections (2). In the USA, the incidence of NIC varies between 3 and 10 % for very low birth weight neonates (VLBW; 1000 – 1500 g) and between 6 and 20% for extremely low birth weight neonates (ELBW; <1000 g) (1, 3). NIC is a severe illness, with a case-fatality rate up to 30% (4), and carries an additional risk of subsequent neurodevelopmental impairment due to *Candida* spp. meningoencephalitis (4). The significant decrease of NIC incidence since the end of the 2000s is partly explained by the identification and control of its modifiable risk factors (5), such as administration of broad-spectrum antibiotics, presence of a central venous catheter, exclusive parenteral nutrition, and mechanical ventilation (6).

The diagnosis of NIC remains difficult. Only 40% of neonates suspected of NIC have fever, and other clinical manifestations of neonatal sepsis such as tachycardia, poor perfusion, respiratory distress, feeding difficulties, and jaundice, are insufficiently specific (7). Laboratory findings such as thrombocytopenia, leukocytosis, and elevated C-reactive protein (CRP) levels are not specific for candidiasis (7-9). NIC can only be proven by culturing *Candida* spp. from a normally sterile site, e.g., blood or cerebrospinal fluid samples (10), but the sensitivity of fungal blood culture may vary from 21 to 71%, as suggested by autopsy-proven invasive candidiasis studies in adults (11). The sensitivity of blood culture is even lower in neonates because the blood sample obtained for culture is often less than 1 ml (12). Moreover, the delay in obtaining a positive culture result might be considered too long, usually 2 to 5 days, to enable early diagnosis and treatment (13). Delays in instituting appropriate antifungal therapy may lead to an increase in morbidity and mortality (14). There is no single gold standard for determining the presence or absence of NIC. Therefore, most clinicians and researchers rely on risk stratification

systems such as European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria, which grade the diagnosis of invasive fungal infection with three levels of probability (proven, probable, and possible).

New biomarkers would be helpful for decision-making in neonates with sepsis. PCR based-assays for *Candida* have shown promising results, but clinical studies in neonates are scarce (15, 16). Serum (1,3)-Beta-D-Glucan (BDG), a cell wall component of most pathogenic fungi, has been introduced in the revised version of EORTC/MSG diagnostic criteria for invasive candidiasis in 2008 (10). Presence of BDG in the serum (Fungitell® > 80 ng/ml) allows the clinician to classify the patient as having "probable" instead of "possible" systemic fungal infection. Results of serum BDG levels can be obtained in less than 24 hours, and the amount of blood needed for the measurement of BDG is acceptable for neonates (< 100 µL).

In a recent meta-analysis in adults (17), BDG showed a summary sensitivity and specificity for detecting invasive fungal infection of 80% (95CI: 77% - 82%) and 82% (81% - 83%), respectively. BDG seems able to rule out invasive fungal infection in children and adults (18-20), but there is limited data for its use in neonates, and a diagnostic accuracy systematic review and meta-analysis is lacking. The accuracy of BDG deserves being investigated in neonates because of specific risk factors and epidemiology of *Candida* infections, and higher rates of dissemination and end-organ damage in this population (21). This study aimed to determine the diagnostic accuracy of BDG in NIC.

METHODS

This systematic review was reported following the *Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies* statement (**Appendix 1**) (22). This study was registered

with PROSPERO (CRD42018089545).

Eligibility criteria

We included published and unpublished studies which compared BDG (Fungitell® assay, Associates of Cape Cod, Inc., Falmouth, MA) with any reference standard that defined groups of patients with ordinal certainty of NIC (e.g., EORTC/MSG criteria) and included fungal blood culture. We included prospective and retrospective cross-sectional studies, randomised controlled trials, and case–control studies. We excluded studies in which another diagnostic kit than Fungitell® (e.g., GKT®) was evaluated, and case series with less than 10 participants. We included only studies reported in English, French, and Spanish.

Literature search strategy

The search strategy was developed in consultation with an information specialist (RS). We searched Medline via Pubmed using the search strategy described in **Appendix 2**. The search was adapted to search Embase via Ovid. We did not use methodological filters to identify diagnostic studies because such filters may result in the omission of relevant studies (23), but used the Cochrane Neonatal group's standard search strategy for the neonatal population (available at neonatal.cochrane.org). We hand-searched reference lists of included studies, of guidelines for the management of candidiasis, and of previous reviews about BDG (17, 19, 24-27). We also searched for eligible articles using the "similar articles" function in PubMed (20 first related articles of each included article). We used Google Scholar to search for newer reports citing included articles. Conference abstracts and proceedings listed in Embase were included in our screening. Furthermore, one author (JFC) additionally searched ClinicalTrials.gov and browsed through all available issues of the Fungitell® Bulletin

(www.acciusa.com/clinical/fungitell/Fungitell_Bullitens.html), a quarterly newsletter from the test manufacturer (March 2010 until March 2019).

Study selection

First, two review authors (AO, JFC) independently excluded studies that were not related to NIC or BDG on the basis of the titles and abstracts identified by the search strategy. Then, two review authors (AO, JFC) retrieved the full text of relevant articles and independently evaluated them for inclusion using predetermined inclusion criteria.

Data extraction and management

For each included study, the following set of data was extracted using a pro forma: first author, year of publication or presentation, inclusion criteria, study setting, study design, reference standard used, number of participants, participant characteristics, threshold values for BDG investigated in the study report, data needed to assess methodological quality, and numbers to construct 2 x 2 tables (number of true positives, true negatives, false positives, and false negatives) at predefined positivity thresholds of 80 pg/ml (as recommended by the manufacturer) and 120 pg/ml. When study authors did not provide such numbers, we either calculated these numbers based on the reported estimates of sensitivity and specificity, or attempted to contact the authors to obtain additional information. According to the clinical and biological data available for each study, we reclassified participants according to the following three risk categories: proven NIC, probable NIC, and possible or excluded NIC. Data extraction was performed by one author (AO) and checked by a second (JT). One review author (JFC) acted as arbiter in case of discrepancies.

Assessment of methodological quality

Methodological quality assessment involved the use of the *Quality Assessment of Diagnostic Accuracy Studies-2* (QUADAS-2) tool (28). For each study, we scored risk of bias and concerns regarding applicability according to the four domains of QUADAS-2 (i.e., patient selection, index test, reference standard, and flow and timing).

Statistical analysis and data synthesis

Based on data from the 2 x 2 tables, we displayed estimates of sensitivity and specificity on forest plots and in the receiver-operating characteristic (ROC) space to represent the variability in diagnostic test accuracy within and between studies. We used Stata/SE version 13 (StataCorp, College Station, TX) to fit the hierarchical bivariate model (29), which allows for calculating summary estimates of sensitivity and specificity and the associated 95% confidence intervals, at prespecified positivity thresholds of 80 pg/ml and 120 pg/ml.

In our main analysis, participants with proven and probable NIC were considered as having NIC (reference standard positives), while participants with possible or excluded NIC were considered as not having NIC (reference standard negatives). In an additional analysis, we estimated the sensitivity of BDG to detect proven NIC cases, at a prespecified threshold of 80 pg/ml. We also carried out a sensitivity analysis by including only studies judged at low risk of bias.

RESULTS

Results of the search

The database literature search was performed on July 25, 2019 (last update). A chart displaying the flow of studies through the review appears in **Figure 1**. Searching in Medline and Embase identified 1697 unique records. We excluded a total of 1674 reports on the basis of their title, abstract or both. After evaluating the full text of 23 studies, we excluded 18 (**Appendix 3**). Additional searches allowed us to include three other studies (30-32). Overall, we included eight studies in our systematic review and meta-analysis (16, 30-36).

Characteristics of included studies

Table 1 summarises the main characteristics of the included studies. Four originated from France, one from Italy, one from Spain, one from South Africa, and one from Egypt. Five of them were retrospective (and among them, two were case–control studies), one was a single centre prospective study, and two were multicenter prospective cohort studies. A total of 465 participants were included across studies (range 13 to 155), with a median gestational age of 30 weeks of gestation (WG) (range 27 to 31.7) and a median birth weight of 1027.5 g (range 991.1 to 1811.4). Inclusion criteria varied (see detailed study characteristics in **Appendix 4**). Each author used case definitions derived from EORTC/MSG criteria (10), but there was substantial heterogeneity in risk stratification systems from a study to another; only the definition of proven cases was homogeneous (i.e., *Candida*-positive culture from a normally sterile site; see **Table 2**). Authors used various rules combining host, clinical and mycological criteria to define NIC status. Some of them used additional biological criteria such as CRP level, leukocytosis, and platelet count (33, 35). NIC risk factors and colonisation by *Candida* spp. were often taken into consideration

when defining probable NIC (31-35). The number of proven NIC cases ranged from 4 to 15 per study, for a total of 60.

Methodological quality of included studies

Table 3 summarises quality assessment of the studies included in the review (see detailed quality assessment in **Appendix 4**). Two of the studies were judged as being of low overall risk of bias (≥ 3 QUADAS-2 items) (31, 34). Five studies avoided clinical selection of participants and therefore included a representative spectrum of patients. Interpretation of the results of the reference standard was made with blinding of the result of the BDG in 3 of 8 studies. There was low concern about applicability for patient selection, the index test, and the reference standard, except for the two case–control studies (30, 36).

Summary estimates of accuracy

The meta-analysis included eight studies encompassing 465 participants in total. At an 80 pg/ml positivity threshold, sensitivity varied from 75% to 100% and specificity from 47% to 100% (**Figure 2A**); bivariate meta-analysis showed a summary sensitivity of 89% (80% - 94%) and a summary specificity of 60% (54% - 66%) (**Figure 3A**). At a 120 pg/ml positivity threshold, sensitivity varied from 65% to 100% and specificity from 66% to 100% (**Figure 2B**); the summary estimates of sensitivity and specificity of BDG were 81% (71% - 88%) and 80% (67% - 88%), respectively (**Figure 3B**).

Additional analyses

When restricting the meta-analysis to patients with proven NIC, and using a positivity threshold of 80 pg/ml, BDG had a summary sensitivity of 99% (93% - 100%); only 2 out of 60 cases of proven NIC would have been missed by BDG (**Appendix 5**).

We initially planned a sensitivity analysis restricted to studies judged at low risk of bias in at least three QUADAS-2 domains, but only two studies fulfilled this quality criterion. We investigated the impact of study quality by restricting the meta-analysis to non-case–control studies. Compared with the overall results (summary sensitivity and specificity of 89% and 60%, respectively), at a threshold of 80 pg/ml, sensitivity was lower and specificity was stable when considering exclusively the six non-case–control studies (summary sensitivity and specificity of 85% and 59%, respectively).

DISCUSSION

Main findings

NIC is a serious illness for which diagnosis is particularly difficult. In this meta-analysis, including eight studies encompassing 465 patients, summary sensitivity and specificity of BDG were 89% (80% - 94%) and 60% (54% - 66%) at an 80 pg/ml threshold, and 81% (71% - 88%) and 80% (67% - 88%) at a 120 pg/ml threshold. There were substantial variations in inclusion criteria and risk stratification systems across studies. In an additional analysis, we found that BDG was able to detect 99% of proven NIC cases when using an 80 pg/ml positivity threshold. In non-case–control studies, the sensitivity of BDG was lower, and specificity was stable (85% and 59%, respectively).

Comparison with previous findings

The accuracy of BDG for detecting invasive fungal infection was studied in pediatric patients with oncologic and hematologic malignancies (18). Using an 80 pg/ml threshold, the sensitivity and specificity of BDG were 42% and 71%, respectively. Thus, BDG seems less sensitive in this type of patients than in neonates. Guitard *et al.* explained this lower sensitivity by the frequent use of empiric and pre-emptive echinocandins prior to BDG measurement. The authors hypothesised that echinocandins might cause false-negative BDG results by inhibiting the activity of enzyme BDG-synthase (18).

In adults, two recent systematic reviews with meta-analysis evaluated the diagnostic accuracy of BDG for detecting invasive fungal infection. At an 80 pg/ml threshold, sensitivity and specificity were 77% and 85% (19), respectively, and 78% and 81% (24). Again, BDG seems more sensitive in neonates, at the expense of specificity. False-positive BDG results could be explained by cellulose-containing membranes used in hemodialysis, surgical glucan-containing gauzes, administration of human blood products such as albumin and intravenous immunoglobulins, treatment with certain antibiotics such as piperacillin-tazobactam, and glucan-containing test tubes used the laboratory (11, 37). Of note, no false positivity related to the preterm neonatal status *per se* has been reported.

Three studies that evaluated BDG in neonates were excluded from this review because they didn't use the Fungitell® assay but a different commercial kit (i.e., GKT® and Dynamiker Fungus® assays)(38-40). Reported positivity thresholds for the GKT® assay were lower than for Fungitell® (14 pg/ml for Liu *et al.* and 10 pg/ml for Zhao *et al.*). Sensitivity and specificity of the GKT® assay were 75% and 91%, respectively, in Liu's study, and 68% and 75%, respectively, in Zhao's. The reported threshold for the Dynamiker fungus® assay was 99 pg/ml, which seems in the same range than that of Fungitell®; its sensitivity and specificity were 64% and 95 %, respectively (40).

Strengths and limitations

In this review, two reviewers independently performed study selection, but data extraction and quality assessment was performed by one reviewer and checked by a second. Studies included in this review are not exempt from methodological weaknesses, and results should be considered with caution. Also, the number of studies (n = 8) and participants (n = 465) are rather low. This led to imprecision in meta-analysis summary estimates and impeded further analysis of potential sources of heterogeneity between studies, for example by use of meta-regression. Also, because of the lack of reported data, we were only able to analyse the accuracy of BDG at two prespecified thresholds (i.e., 80 pg/ml and 120 pg/ml).

Implications

At an 80 pg/ml threshold, BDG has a high sensitivity for detecting proven NIC (99%). When focusing on "proven and probable NIC", a less stringent outcome, sensitivity dropped to 89% in our meta-analysis, which means that one out of ten patients with "proven and probable NIC" would be missed in a strategy relying only on BDG. Although BDG has a high sensitivity for proven NIC, it has only moderate accuracy for "proven and probable NIC" combined. As a consequence, BDG should not be used as a stand-alone test to rule out NIC and eventually stop empirical antifungal treatment. We probably should incorporate BDG in a clinical decision rule combining clinical, biological and radiological information, as suggested by Benjamin *et al.* (4). BDG could also be incorporated in a clinical scoring system such as the "Candida Score" developed by Leon *et al.* to assess the risk of invasive candida infection in non-neutropenic adults (41). We are not aware of studies assessing the clinical usefulness of BDG-based compared to such risk-based approaches.

There was high variability in reference standards and case definitions across studies. The presence of such variability highlights that the EORTC/MSG criteria seem challenging to apply in neonates. We suggest using a simplified rule-based risk stratification system relying on the presence of signs and symptoms of neonatal sepsis, risk factors for invasive candidiasis, evidence of *Candida* colonisation, and *Candida* being cultured from a normally sterile site (**Table 4**). We believe a more consensual risk stratification system would increase homogeneity in case definition and help interpreting results from future studies.

More prospective multicenter cohort studies with blinding of test readers are needed. Such studies should not focus on BDG alone, but should also investigate the value of other biomarkers such as platelet and leukocyte counts, CRP, mannan and anti-mannan antibodies, and *Candida* PCR, for example. This would allow direct comparisons of test performance. It could also be interesting to measure BDG systematically and repeatedly in the serum and cerebrospinal fluid of newborns with NIC because BDG monitoring might allow reducing the length of antifungal therapy.

Conclusions

BDG seems a promising biomarker in the management of newborns with suspected NIC. Notably, BDG might be helpful in clinical practice to exclude NIC diagnosis. However, it seems too early to recommend its use because of the scarcity of reliable clinical data, heterogeneity in case definitions, and unstable accuracy estimates. Given the current evidence, BDG cannot be used as a stand-alone test to decide whether or not the treatment threshold for NIC is reached. Larger high-quality clinical studies in neonates with sepsis are warranted.

TRANSPARENCY DECLARATION

Protocol: The protocol of this study was registered and published on the PROSPERO website (number CRD42018089545).

Conflicts of interest: None.

Funding: There was no external funding for this study.

Acknowledgements: We thank Drs Cliquennois, Cornu, Mackay, and Goudjil for having shared additional unpublished data.

Access to data: JFC had full access to the data; he is the guarantor for the data.

Authors' contribution: AO and JFC performed study selection. AO and JT performed data extraction. JFC analysed the data. JFC and AO wrote the first draft of the manuscript. RS designed the literature search strategy. JFC and JT coordinated the development of the study, wrote the first draft of the protocol, designed the study, and finalised the manuscript. All authors revised the manuscript for important intellectual content and approved the final version submitted for publication.

TABLES

Table 1. Characteristics of included studies

Study	Country	Inclusion criteria	Study design	N	Median gestational age (weeks)*	Median birth weight (g)*	Risk stratification system (n)**	Fungal species when identified
Cliquennois 2018	France	Neonates suspected of NIC	Prospective single center cohort	61	27.6	1035	Proven NIC (4) Probable NIC (4) Possible NIC (53)	7 *C. albicans* 1 *C. Parapsilosis* 1 unspecified yeast
Cohen 2017	France	Neonates suspected of NIC, with available BDG serum measurements	Retrospective single centre cohort	13	27.3	1020	Proven NIC (5) Probable NIC (4) Possible NIC (4)	4 *C. albicans* 1 *C. glabrata*
Cornu 2018	France	Neonates suspected of late-onset infection, with available BDG serum measurements	Retrospective single centre cohort	38	30	1200	Proven group (4) Probable group (13) Control group (21)	16 *C. albicans* 1 *C. parapsilosis*
Goudjil 2012	France	Neonates suspected of NIC, with available BDG serum measurements	Retrospective single centre cohort	61	28.5	1000	Infected group (18, including 6 proven NIC) Non-infected group (43)	14 *C. albicans* 3 *C. parapsilosis* 1 *C. lusitaniae*
Mackay 2011	South Africa	Neonates suspected of late-onset infection, with high risk of NIC	Prospective multicenter cohort	72	31	1340	Definite fungemia (10) Probable fungemia (9) Possible fungemia (22) No fungemia (31)	4 *C. albicans* 4 *C. parapsilosis* 1 *Saccharomyces cerevisiae*
Montagna 2011	Italy	Preterm neonates with NIC, and non-infected preterm neonates	Retrospective single centre case–control	20	Not reported	1145	Proven *Candida* bloodstream infections (10) Negative controls (10)	6 *C. albicans* 3 *C. parapsilosis* 1 *C. glabrata*
Ramos 2017	Spain	Neonates suspected of late-onset sepsis or meningitis in NICU	Prospective multicenter cohort	155	27	882	IC confirmed (6) IC probable (2) Controls (147)	8 *C. albicans* 1 *Candida spp*
Tabl 2012	Egypt	Neonates with NIC, neonates with bacteremia, and healthy controls***	Retrospective single center case–control	45	Candidemia: 30.4 Bacteremia: 30.5 Control: 31.7	Candidemia: 1018.7 Bacteremia: 991.1 Control: 1811.4	Candidemia (15) Bacteremia (15) Control (15)	Not reported

Abbreviations: WG, weeks of gestation; BDG, Beta-D-Glucan; NIC, neonatal invasive candidiasis; NICU, neonatal intensive care unit. *Gestational age and birth weight reported as median except for Ramos and Tabl (mean). ** According to case definitions used in the original articles. ***In meta-analysis, neonates with bacteremia and controls were grouped as NIC-negatives.

Table 2. Risk stratification systems used across studies

Study	Risk stratification system
Cliquennois 2018	See Table 4
Cohen 2017	See Table 4
Cornu 2018	• **Control group:** neonates who were neither colonised nor infected with any fungal pathogen; • **Probable group:** neonates who had probable invasive yeast infection (IYI) suspected from clinical and biological criteria; Probable IYI was defined as receipt of more than 5 days of antifungal therapy and presence of yeast in urine (urine bag) or digestive (stool and anal swab) colonisation with clinical or biological (white blood cell count, C-reactive protein [CRP])) symptoms of sepsis. • **Proven group:** neonates who had proven IYI. Proven IYI was defined as a positive culture for a yeast species from a normally sterile body site except urine obtained with a urine bag.
Goudjil 2012	• **Infected group:** patients with systemic infection, including a *Candida*-positive culture from a usually sterile body site: candidemia (only blood cultures positive for *Candida* spp.), disseminated candidiasis (blood, urine, CSF, peritoneal or pleural spaces, bone, joint or ocular sites positive for *Candida* spp.), renal candidiasis (urine cultures are positive for *Candida* spp. with ultrasound evidence of fungal lesions in the kidney); • **Non-infected group:** patients with symptoms of infection but without any positive fungal culture from sterile body sites.
Mackay 2011	• **No fungemia:** a high index of suspicion for fungemia including 3 or more risk factors; blood culture negative; white cell count (WCC), platelet count (<100 000/mm^3) and C-reactive protein (CRP) within normal limits; no evidence of colonisation with a fungal organism; or definite bacterial sepsis; • **Possible fungemia:** a high index of suspicion for fungemia including 3 or more risk factors; blood cultures negative; no evidence of colonisation; two or more of the following present: abnormal WCC, low platelet count or elevated CRP (>10); • **Probable fungemia:** a high index of suspicion for fungemia including 3 or more risk factors and 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/mm^3) or elevated CRP (>10); • **Definite fungemia:** positive culture for a fungal organism from a normally sterile site (including blood or tissue culture).
Montagna 2011	• **Proven *Candida* bloodstream infections** • **Negative controls**
Ramos 2017	• **Invasive candidiasis (IC) confirmed:** an episode was defined as IC confirmed if a culture of a sterile sample was found to be positive for *Candida* spp. or if a *Candida* species was detected during the autopsy of a deceased patient. Likewise, an episode was considered IC confirmed when endophthalmitis candidiasis was diagnosed by direct examination of the fundus of the eye or if fungus balls were observed by abdominal ultrasound. • **IC probable:** this designation was used for patients who tested negative for *Candida* spp. in a culture derived from blood, cerebrospinal fluid (CSF), or other sterile fluid samples but for whom (i) improvement was seen upon treatment with an antifungal(s) and/or (ii) the episode corresponded to a true IC (according to the clinician's criteria and based on the evolution) and/or (iii) the image from an echocardiogram was consistent with *Candida* endocarditis or a cerebral ultrasound showed micro-abscesses consistent with IC. In addition to fulfilling any of the criteria described above, one of the following conditions had to be met: colonisation by *Candida* spp. was detected by isolation of *Candida* spp. from stool, urine, bronchial aspirate, or gastric samples or a clinical diagnosis of *Candida* dermatitis was made; thrombocytopenia (platelet count of 100,000/mm^3) was diagnosed; or, after an episode of sepsis, a complication such as meningitis, endocarditis, or pyelonephritis appeared as a result of the dissemination of *Candida* spp. This condition was determined by clinical judgment. Mucosal candidiasis in the absence of clinical signs of IC was not considered to represent IC.
Tabl 2012	• **Candidemia group**: culture-proven candidemia • **Bacteremia group**: culture-proven bacteremia • **Control group**: healthy preterm neonates

Table 3. Risk of bias and concerns regarding applicability of included studies

	Risk of bias				Applicability concerns		
Study	**Patient selection**	**Index test**	**Reference standard**	**Flow and timing**	**Patient selection**	**Index test**	**Reference standard**
Cliquennois 2018	Low	Low	Low	Low	Low	Low	Low
Cohen 2017	High	High	High	High	Low	Low	Low
Cornu 2018	Low	Low	High	Unclear	Low	Low	Low
Goudjil 2012	Low	Low	Low	Unclear	Low	Low	Low
Mackay 2011	Low	Unclear	Unclear	Unclear	Low	Low	Low
Montagna 2011	High	High	High	High	High	Low	High
Ramos 2017	Low	Low	Unclear	High	Low	Low	Low
Tabl 2012	High	High	Unclear	High	High	Low	High

Table 4. Proposed simplified risk stratification system for neonatal invasive candidiasis

NIC	≥ One criterion for neonatal sepsis [a]	≥ One risk factor for NIC [b]	Evidence of *Candida* colonisation [c]	*Candida* positive culture from a normally sterile site [d]
Proven	Yes	+/-	+/-	Yes
Probable	Yes	Yes	Yes	No
Possible	Yes	Yes	No	No

[a] Fever, cardiovascular instability (tachycardia, bradycardia, poor perfusion), respiratory symptoms (respiratory distress, apnea, oxygen desaturation), neurological symptoms (lethargy, irritability, seizures), feeding difficulties (vomiting, abdominal distension), jaundice;
[b] Term < 28 WG, Neonatal weight < 1000 g, central venous catheter, exclusive parenteral nutrition, broad-spectrum antibiotics, mechanical ventilation with endotracheal intubation, post-natal corticosteroids, gastrointestinal tract disease (abdominal surgery, necrotising enterocolitis, spontaneous intestinal perforation);
[c] At least two positive sites among skin, stools or anal swab, mouth, respiratory tract, and urine without ultrasound findings suggestive of fungal lesions;
[d] Blood, cerebrospinal fluid, bone or joint, pleural or pericardial or peritoneal fluid, urine with ultrasound findings suggestive of fungal lesions, such as fungus balls, intraocular fluids and tissues.

FIGURE CAPTIONS

Figure 1. Flow diagram of studies in the review.

Figure 2. Forest plots of (1,3)-Beta-D-Glucan sensitivity and specificity for detecting neonatal invasive candidiasis. TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative.

Figure 3. Summary ROC plot of (1,3)-Beta-D-Glucan sensitivity and specificity for neonatal invasive candidiasis (n = 8). Each study cohort is represented by an empty square. The filled circle is the pooled summary estimate for sensitivity and specificity. The dotted line represents the 95% confidence region for the summary operating point.

Figure 1

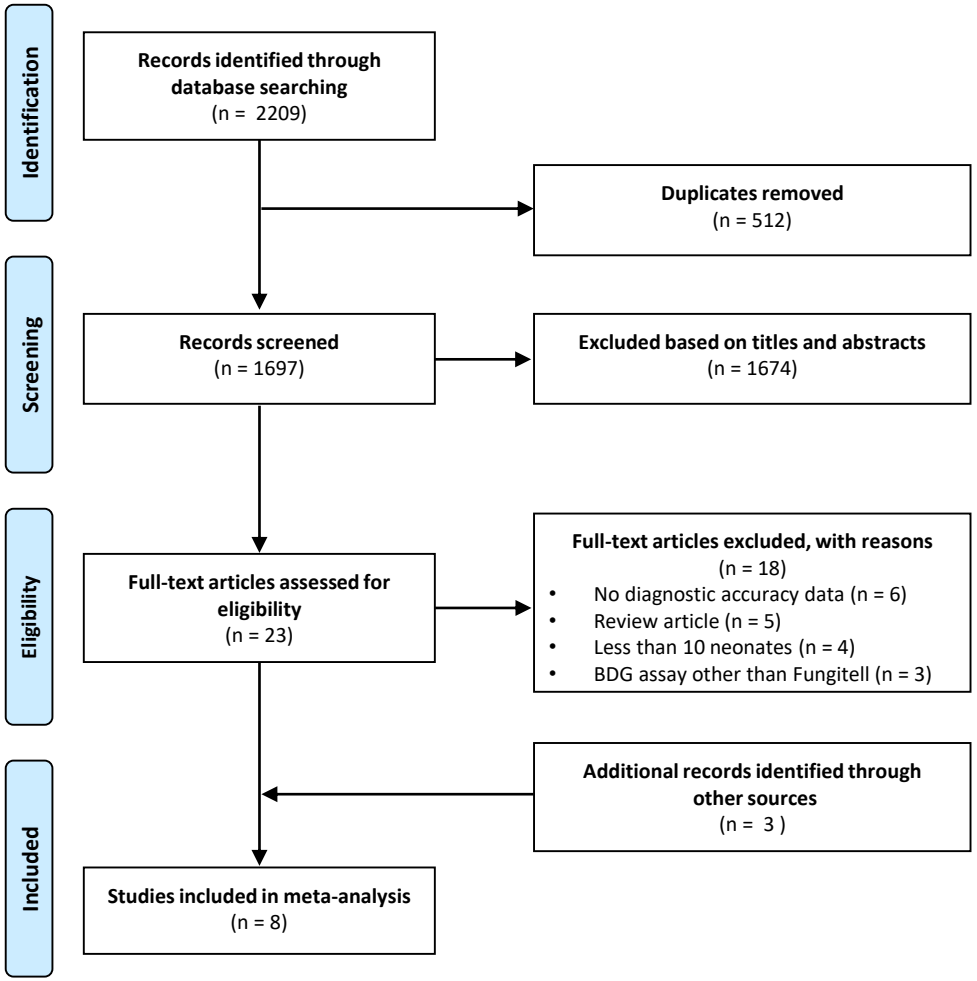
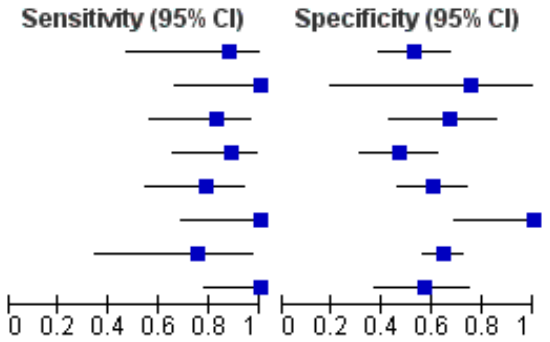

Identification
Records identified through database searching
(n = 2209)
Duplicates removed
(n = 512)
Screening
Records screened
(n = 1697)
Excluded based on titles and abstracts
(n = 1674)
Eligibility
Full-text articles assessed for eligibility
(n = 23)
Full-text articles excluded, with reasons
(n = 18)
• No diagnostic accuracy data (n = 6)
• Review article (n = 5)
• Less than 10 neonates (n = 4)
• BDG assay other than Fungitell (n = 3)
Additional records identified through other sources
(n = 3)
Included
Studies included in meta-analysis
(n = 8)

Figure 2

2A. 80 pg/ml threshold

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Cliquennois 2018	7	25	1	28	0.88 [0.47, 1.00]	0.53 [0.39, 0.67]
Cohen 2017	9	1	0	3	1.00 [0.66, 1.00]	0.75 [0.19, 0.99]
Cornu 2018	14	7	3	14	0.82 [0.57, 0.96]	0.67 [0.43, 0.85]
Goudjil 2012	16	23	2	20	0.89 [0.65, 0.99]	0.47 [0.31, 0.62]
Mackay 2011	15	21	4	32	0.79 [0.54, 0.94]	0.60 [0.46, 0.74]
Montagna 2011	10	0	0	10	1.00 [0.69, 1.00]	1.00 [0.69, 1.00]
Ramos 2017	6	52	2	95	0.75 [0.35, 0.97]	0.65 [0.56, 0.72]
Tabl 2012	15	13	0	17	1.00 [0.78, 1.00]	0.57 [0.37, 0.75]



2B. 120 pg/ml threshold

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Cliquennois 2018	7	16	1	37	0.88 [0.47, 1.00]	0.70 [0.56, 0.82]
Cohen 2017	8	1	1	3	0.89 [0.52, 1.00]	0.75 [0.19, 0.99]
Cornu 2018	11	3	6	18	0.65 [0.38, 0.86]	0.86 [0.64, 0.97]
Goudjil 2012	15	11	3	32	0.83 [0.59, 0.96]	0.74 [0.59, 0.86]
Mackay 2011	14	18	5	35	0.74 [0.49, 0.91]	0.66 [0.52, 0.78]
Montagna 2011	10	0	0	10	1.00 [0.69, 1.00]	1.00 [0.69, 1.00]
Ramos 2017	6	47	2	100	0.75 [0.35, 0.97]	0.68 [0.60, 0.75]
Tabl 2012	13	1	2	29	0.87 [0.60, 0.98]	0.97 [0.83, 1.00]

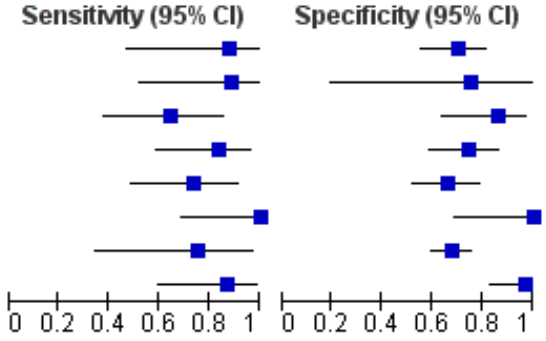
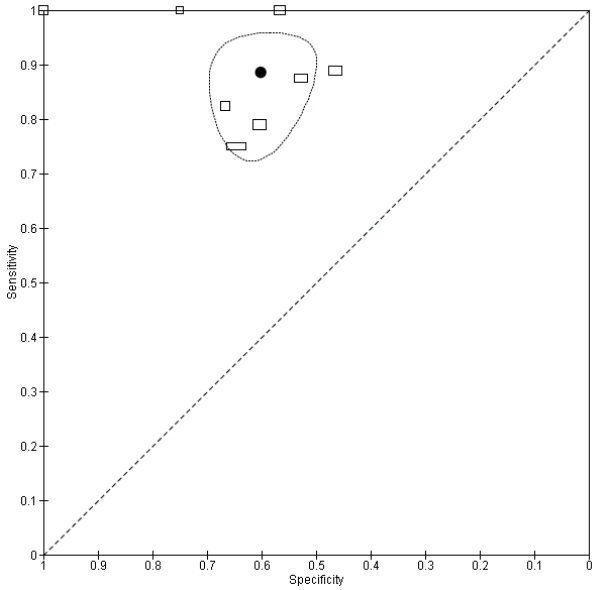
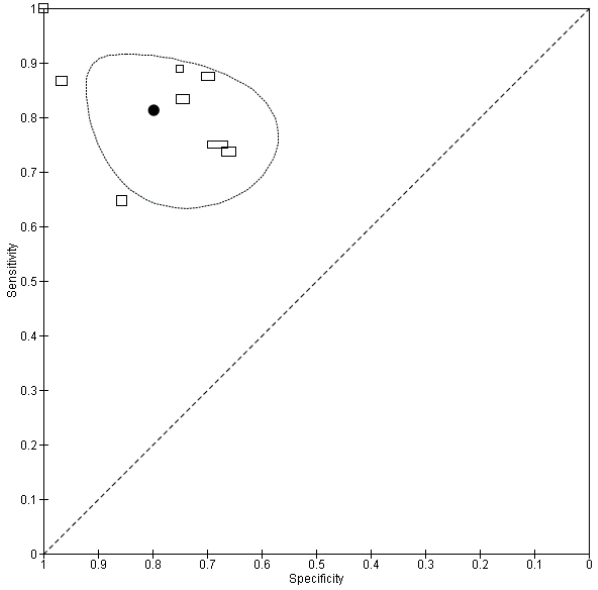


Figure 3

3A. 80 pg/ml threshold



3B. 120 pg/ml threshold



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