Conclusion: (1,3)-β-D-glucan assay has a limited sensitivity with excellent specificity and negative predictive value, which allow its use as an aid in exclusion of invasive neonatal fungal infection. Accurate diagnosis and therapeutic decisions should be based on combining (1,3)-β-D-glucan assay with other clinical, radiological, and microbiological findings.

PALAVRAS-CHAVE
(1,3)-β-D-glucano;
Candidíase invasiva;
Sepse neonatal

O papel do (1.3)-β-D-Glucano no Soro no diagnóstico precoce de infecções fúngicas invasivas em uma unidade de terapia intensiva neonatal

Resumo
Objetivos: Estudar o padrão microbiológico das culturas de sepse neonatal de início tardio e avaliar o desempenho diagnóstico do nível de (1,3)-β-D-glucano no soro para diagnóstico precoce de fungemia invasiva em neonatos de alto risco internados em uma unidade de terapia intensiva neonatal.
Métodos: Ensaio clínico multicêntrico prospectivo conduzido em neonatos internados em uma unidade de terapia intensiva neonatal com suspeita de sepse de início tardio que estavam em risco de infecções fúngicas invasivas no hospital universitário infantil de Almançora e no hospital geral de Almançora entre março de 2014 e fevereiro de 2016.
Resultados: 77 neonatos recém-nascidos com risco de infecção fúngica invasiva foram classificados, com base na hemocultura, eg136e:T/GS1gsBT/F41Tf.000200-.0002395.5605615.8168Tm()TjET/

to assess the microbiological pattern of neonatal LOS and the diagnostic performance of BG for early diagnosis of IFI in high-risk infants admitted to NICUs.

Subjects and methods

This was a prospective multi-center cohort study conducted in NICUs of the Mansoura University Children's Hospital and of the Mansoura General Hospital from March 2014 to February 2016.

Ethics

The Research Ethics Committee of Mansoura Faculty of Medicine approved the study, and written informed consents were obtained from the parents of all neonates included in the study.

Included subjects

Neonates with clinically suspected LOS (sepsis after 72 h from birth) who were at high risk of IFI were included. Clinical manifestations suggestive of neonatal sepsis were defined in accordance with the Brazilian National Health Surveillance Agency (ANVISA) criteria, in which neonatal sepsis was defined as a systemic response, without any other recognized cause than infection, associated with at least two or more of the following signs and symptoms: thermal instability, apnea, worsening of respiratory discomfort, hemodynamic instability, bradycardia, feeding intolerance, glucose intolerance, hypoactivity, and lethargy.[14]

Patients were considered at high risk for IFI if they had three or more of the following criteria: Low birth weight (<2500 g), hospitalization for >3 weeks, prolonged mechanical ventilation (>1 week), systemic antibiotic exposure >72 h, postoperative patients, abdominal wall defects, central venous catheter, arterial catheter >72 h, total parenteral nutrition administration, and persistent severe thrombocytopenia despite second line antibiotics administration.[13]

Excluded subjects

Neonates who received systemic antifungal drugs (prophylactic or therapeutic), intravenous immunoglobulin, albumin, plasma protein, and amoxicillin-clavulanic acid antibiotic were excluded from the study.

All recruited infants underwent LOS investigations: complete blood count, total and differential; quantitative C-reactive protein (CRP); and full septic workup, including blood, urine, CSF cultures, as well as serum BG level. Other laboratory and radiological investigations were performed according to the decision of the attending physician.

Neonates included were categorized according to their blood culture results into three groups. The no fungemia group included 41 neonates with blood culture-proven bacterial sepsis. The definite fungemia group included 11 neonates with blood culture-proven fungemia. Finally, the suspected fungemia group included 25 neonates with

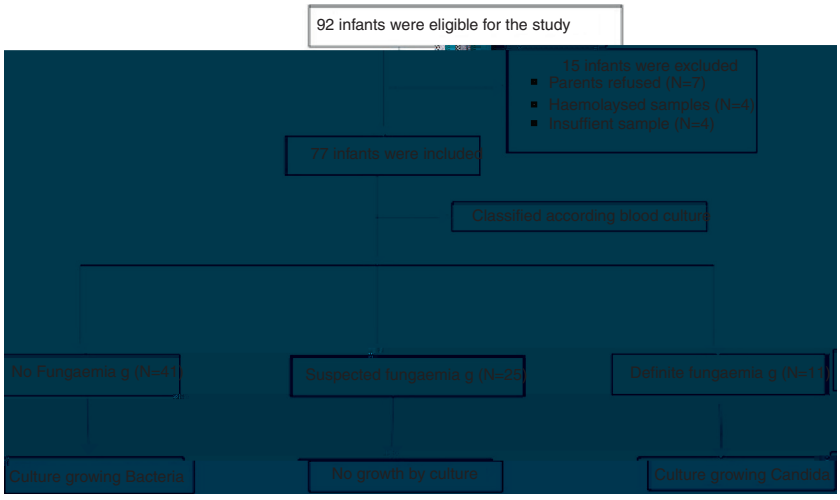

Figure 1 Study flowchart.

Results

During the study period, a total of 77 neonates with LOS at a high risk of IFI were included; the stratification of patients into groups is demonstrated in the study flow chart (Fig. 1). Baseline characteristics of studied groups revealed that gestational age and birth weight were significantly lower in definite fungemia patients when compared with those in the no fungemia and suspected fungemia groups. Postnatal age, Cesarean section delivery, and male gender were significantly higher in definite fungemia patients when compared with no fungemia and suspected fungemia patients (Table 1). Low birth weight, hospitalization >3 weeks, central device, persistent thrombocytopenia, WBC, and CRP were significantly higher in the definite fungemia group when compared with the other two groups, while mechanical ventilation or continuous positive airway pressure (CPAP) >1 week was significantly higher in the definite fungemia group when compared with

Table 1 Demographic, clinical, and laboratory characteristics of the studied groups.

	No fungemia $n = 41$	Suspected fungemia $n = 25$	Definite fungemia $n = 11$	p^a	p^b	p^c	p^d
Gestational age (weeks)	33.41 ± 3.294	33.08 ± 3.511	30 ± 0.256	0.045	0.069	0.037	0.007
Postnatal age (days)	23.76 ± 8.249	21.08 ± 7.205	30.91 ± 6.236	0.003	0.173	0.008	0.001
Birth weight (g)	2100 (850–3900)	1880 (830–4700)	930 (830–3350)	0.038	0.041	0.020	0.018
Male gender	28 (68.3%)	13 (52%)	11 (100%)	0.007	0.187	0.031	0.005
Cesarean section delivery	32 (78%)	19 (76%)	11 (100%)	0.041	0.847	0.047	0.035
Prolonged rupture of membranes	28 (68.3%)	18 (72%)	7 (63.6%)	0.892	0.751	0.770	0.616
Low birth weight (<2500							

Regarding the BG assay in the present study, in the no fungemia group, 23 were found negative, 14 were equivocal, and four were positive. In turn, in the definite fungemia group, seven were found positive, three were equivocal, and one was negative. False-positive BG reactions are suspected to occur in patients treated with intravenous immunoglobulins, albumin, coagulation factors, and plasma protein fraction manufactured by certain fungal vendors, or antibiotics derived from fungal sources as amoxicillin-clavulanic acid, which were all excluded in our study. Patients' exposure to gauze or materials containing glucans during surgery, mucosal damage from chemotherapy or radiotherapy, cross-experimental contamination with BG due to excess manipulation of a sample, certain streptococci, and *P. aeruginosa* may also cause false positive reactivity.[23,24] Moreover, Zheng et al. reported that postnatal corticosteroids therapy can cause false positive BG result.[25] In the present study, two neonates from the no fungemia group had history of recent surgery, with possible exposure to materials that contained glucans. In addition, *P. aeruginosa* was isolated from three neonates within the no fungemia group, which may explain their false positive reactivity. In the suspected fungemia group, nine of the neonates presented positive BG reaction, which might be explained by the low sensitivity of blood culture in the diagnosis of invasive candidiasis, as only 50% of invasive candidiasis are blood-culture positive.[9] Furthermore, fungi other than *Candida*, such as *Aspergillus*, may be the cause of positive BG in these patients, since blood cultures are almost always negative in disseminated aspergillosis.[26] Finally, the false negative result of BG in one patient from the definite fungemia group may be explained by IFI at an early stage.

The sensitivity, specificity, PPV, NPP, and accuracy of the BG assay in the present study at the cut-off value of 95 pg/mL recommended by the

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Motta M, Zini A, Regazzoli A, Garzoli E, Chirico G, Caimi L, et al. Diagnostic accuracy and prognostic value of the CD64 index in very low birth weight neonates as a marker of early-onset sepsis. Scand J Infect Dis. 2014;45:433–9.
2. Makhoul IR, Kassis I, Smolkin T, Tamir A, Sujov P. Review of 49 neonates with acquired fungal sepsis: further characterization. Pediatrics. 2001;107:61–6.
3. Filioti J, Spiroglou K, Panteliadis CP, Roilides E. Invasive candidiasis in pediatric intensive care patients: epidemiology, risk factors, management, and outcome. Intensive Care Med. 2007;33:1272–83.
4. Schuchat A, Zywicki SS, Dinsmoor MJ, Mercer B, Romaguera J, O'Sullivan MJ, et al. Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study. Pediatrics. 2000;105:21–6.
5. Kelly MS, Benjamin DK, Smith PB. The epidemiology and diagnosis of invasive candidiasis among premature infants. Clin Perinatol. 2015;42:105–17.
6. Benjamin DK Jr, Stoll BJ, Fanaroff AA, McDonald SA, Oh W, Higgins RD, et al. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics. 2006;117:84.
7. Arnon S, Litmanovitz I. Diagnostic tests in neonatal sepsis. Curr Opin Infect Dis. 2008;21:223–7.
8. Calley JL, Warris A. Recognition and diagnosis of invasive fungal infections in neonates. J Infect. 2017;74:S108–13.
9. Clancy CJ, Nguyen MH. Finding the missing 50% of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013;56:1284–92.
10. Kedzierska A, Kochan P, Pietrzyk A, Kedzierska J. Current status of fungal cell wall components in the immunodiagnostics of invasive fungal infections in humans: galactomannan, mannan and (1→3)-beta-D-glucan antigens. Eur J Clin Microbiol Infect Dis. 2007;26:755–66.
11. Goudjil S, Kongolo G, Dusol L, Imestouren F, Cornu M, Leke A, et al. (1-3)-β-D-Glucan levels in candidiasis infections in the critically ill neonate. J Matern Fetal Neonatal Med. 2013;26:44–8.
12. Zhao D, Qiu G, Luo Z, Zhang Y. Platelet parameters and (1, 3)-β-D-glucan as a diagnostic and prognostic marker of invasive fungal disease in preterm infants. PLOS ONE. 2015;10:0123907.
13. Mackay CA, Ballot DE, Perovic O. Serum 1,3-betaD-glucan assay in the diagnosis of invasive fungal disease in neonates. Pediatr Rep. 2011;3:e14.
14. De Assis Meireles L, Vieira AA, Costa CR. Evaluation of the neonatal sepsis diagnosis: use of clinical and laboratory parameters as diagnosis factors. Rev Esc Enferm USP. 2011;45:33–9.
15. De Vlieger G, Lagrou K, Maertens J, Verbeken E, Meersseman W, Van Wijngaerden E. Beta-d-glucan detection as a diagnostic test for invasive aspergillosis in immunocompromised critically ill patients with symptoms of respiratory infection: an autopsy-based study. J Clin Microbiol. 2011;49:3783e7.
16. Labib AZ, Mahmoud AB, Eissa NA, El Gendy FM, Soliman MA, Aly AA. Early diagnosis of neonatal sepsis: a molecular approach and detection of diagnostic markers versus conventional blood culture. Int J. 2013;4:77–85.
17. Verma P, Berwal PK, Nagaraj N, Swami S, Jivaji P, Narayan S. Neonatal sepsis: epidemiology, clinical spectrum, recent antimicrobial agents and their antibiotic susceptibility pattern. Int J Contemp Pediatr. 2015;2:176–80.
18. Al-Shamahy HA, Sabrah AA, Al-Robasi AB, Naser SM. Types of bacteria associated with neonatal sepsis in Al-Thawra University Hospital, Sana'a, Yemen, and their antimicrobial profile. Sultan Qaboos Univ Med J. 2012;12:48–54.
19. Hornik CP, Fort P, Clark RH, Watt K, Benjamin DK Jr, Smith PB, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum Dev. 2012;88:69–74.
20. Cotten CM, McDonald S, Stoll B, Goldberg RN, Poole K, Benjamin DK Jr. The association of third-generation cephalosporin use and invasive candidiasis in extremely low birth-weight infants. Pediatrics. 2006;118:717–22.
21. Lee JH, Hornik CP, Benjamin DK Jr, Herring AH, Clark RH, Cohen-Wolkowiez M, et al. Risk factors for invasive candidiasis in infants >1500 g birth weight. Pediatr Infect Dis J. 2013;32:222–6.
22. Hope WW, Castagnola E, Groll AH, Roilides E, Akova M, Arendrup MC, et al. ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: prevention and management of invasive infections in neonates and children caused by *Candida* spp. Clin Microbiol Infect. 2012;18:38–52.
23. Pickering JW, Sant HW, Bowles CA, Roberts WL, Woods GL. Evaluation of a (1→3)-beta-D-glucan assay for diagnosis of invasive fungal infections. J Clin Microbiol. 2005;43:5957–62.
24. Mennink-Kersten MA, Ruegebrink D, Verweij PE. *Pseudomonas aeruginosa* as a cause of 1,3-beta-D-glucan assay reactivity. Clin Infect Dis. 2008;46:1930–1.
25. Zheng F, Zha H, Yang D, Deng J, Zhang Z. Diagnostic values and limitations of (1,3)-β-D-glucans and galactomannan assays for invasive fungal infection in patients admitted to pediatric intensive care unit. Mycopathologia. 2017;182:331–8.
26. Arendrup MC, Fisher BT, Zaoutis TE. Invasive fungal infections in the pediatric and neonatal population: diagnostics and management issues. Clin Microbiol Infect. 2009;15:613–24.
27. Liu Y, Chen F, Zhu X, Shen L, Zhang SX. Evaluation of a novel plasma (1,3)-β-d-glucan detection assay for diagnosis of candidemia in pediatric patients. J Clin Microbiol. 2015;53:3017–20.
28. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, et al. Diagnostic accuracy of serum 1,3-β-D-glucan for *Pneumocystis jiroveci* pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J Clin Microbiol. 2012;50:7–15.
29. Hou TY, Wang SH, Liang SX, Jiang WX, Luo DD, Huang DH. The screening performance of serum 1,3-beta-D-glucan in patients with invasive fungal diseases: a meta-analysis of prospective cohort studies. PLOS ONE. 2015;10:e0131602.
30. He S, Hang JP, Zhang L, Wang F, Zhang DC, Gong FH. A systematic review and meta-analysis of diagnostic accuracy of serum 1,3-β-D-glucan for invasive fungal infection: focus on cut off levels. J Microbiol Immunol Infect. 2015;48:351–61.