Serum (1,3)-β-D-Glucan for Screening of Neonatal Fungemia

This study was conducted to identify the mycological pattern, and to calculate the diagnostic accuracy of serum (1,3)-β-D-glucan for screening fungal sepsis in 351 high-risk neonates. *Candida tropicalis* was the most common isolate (*n*=16, 38.1%). At optimum cut-off (47.2 pg/mL), sensitivity and specificity of serum (1,3)-β-D-glucan were 92.9% and 69.9%, respectively.

Keywords: *Candida, Diagnosis, Sepsis.*

Fungal sepsis is a prime cause of neonatal mortality and neurological impairment, particularly in preterm newborns [1,2]. Isolation of fungus from sterile sites remains the gold standard for diagnosis, but it is time-consuming and associated with high false negativity rates. Serum (1, 3)-β-D-glucan (BDG), a component of cell wall of multiple clinically important fungi, is a promising marker of early neonatal fungemia [3]. However, the existing literature suffers from a few lacunae viz., small sample size, inclusion of only preterm newborns, predominantly developed country setting, and only *Candida* species were studied [3,4]. Hence, we conducted this study to find the microbiological pattern of fungal sepsis in neonates at high risk of fungal sepsis, and to assess the diagnostic performance of serum BDG as a screening test in them.

This cross-sectional study was carried out in the neonatal unit (64 bedded Special newborn care unit, 18 bedded Neonatal intensive care unit (level III), and 10 bedded step-down unit) of a public sector tertiary care center between January, 2018, and December, 2021 after taking approval from Institutional ethics committee. Newborns with ≥3 risk factors for fungal sepsis (birth weight <2.5 kg, hospital stay >3 weeks, invasive respiratory support >1 week, antibiotic therapy >72 hours, central venous/ arterial catheter >72 hours, total parenteral nutrition, persistent severe thrombocytopenia despite second line antibiotics) were consecutively enrolled in the study [5]. Newborns with major congenital anomalies, those with parental refusal to participate, and those exposed to prior antifungal therapy, intravenous immunoglobulin, and amoxicillin-clavulanate, were excluded. Cotten, et al. [6] reported approximately 20% prevalence of fungal sepsis in high-risk newborns. Considering a sensitivity and specificity of 80% and 10% error, the required sample size was calculated as 338 (assuming 10% attrition and 5% β error) [7].

Serum BDG level was estimated by spectrophotometry after enrolment. Blood samples of the selected newborns were incubated in BacT/Alert 3D system (*bioMérieux*) at 37°C for 5 days, and subsequently sub-cultured in appropriate medium, if found positive. Other investigations were performed according to clinical indications/ unit protocol. Newborns included in this study did not receive probiotics.

Shapiro-Wilk test was used to check for normal distribution. Chi square test and Mann-Whitney *U* test were used for checking significance of difference between proportions and medians, respectively. Receiver operator characteristic (ROC) curve was generated and Youden index was used to detect the cut-off value for best diagnostic accuracy. SPSS version 16.0 was used for data analysis. $P<0.05$ was taken as statistically significant.

During the study period, 394 admitted newborns met the inclusion criteria, and 43 were excluded (17- received immunoglobulin, 13- received amoxicillin-clavulanate, 5- prior antifungal therapy, 3- inadequate blood sample, 2- refusal of consent 2- major congenital anomaly, 1- left against medical advice). Thus, a total of 351 newborns (48.1% boys) were enrolled in the study. Baseline characteristics of the two groups are shown in **Table I**. Forty-two newborns (12%) developed culture-positive fungal sepsis. *Candida tropicalis* was most common isolate (*n*=16, 38.1%), followed by *Candida albicans* (*n*=13, 31%). All isolates were sensitive to caspofungin and voriconazole, while 37 (88.1%) and 33 (78.6%) isolates were sensitive to amphotericin-B, and fluconazole, respectively. Median (IQR) serum BDG level of newborns who developed culture-positive fungal sepsis was significantly higher than who did not develop culture-positive fungal sepsis [84.6 (26.9) pg/mL vs 34.3 (48.3) pg/mL, $P<0.001$]. Area under the curve of the ROC curve was 0.897 (95% CI: 0.857 - 0.938) (**Fig. 1**). Optimum cut-off was 47.2 pg/mL with a sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio and accuracy, respectively of 92.9% (95% CI: 80.5-98.5%), 69.9% (95% CI: 64.4-75%), 29.6% (95% CI: 25.8-33.6%), 98.6% (95% CI: 96-99.5%), 3.1 (95% CI: 2.6-3.7), 0.1 (95% CI: 0.03-0.3) and 72.6% (95% CI: 67.7-77.2%).

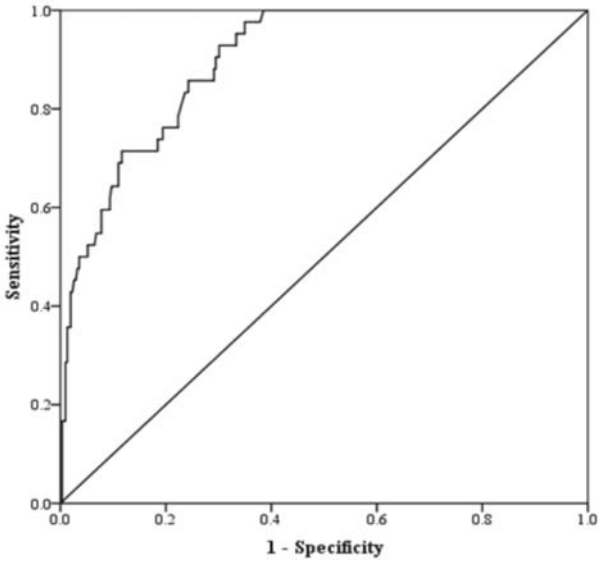

Fig. 1 ROC curve of serum (1,3)–β-D-glucan for discrimination between neonates with and without culture positive fungal sepsis.

Table I Baseline Characteristics of the Study Participants

Variables	Fungal sepsis developed (*n*=42)	Fungal sepsis did not develop (*n*=309)
Birthweight (kg)[a]	1.588 (1.470-2.100)	1.560 (1.280-1.820)
Gestational age (wk)[a]	34.2 (33.1-35)	34.1 (33.2-34.8)
Male sex	22 (52.4)	147 (47.6)
Preterm	41 (97.6)	307 (99.4)
Antenatal corticosteroid[b]	13 (68.4)	104 (70.3)
Multiple pregnancy	12 (28.6)	72 (23.3)
Cesarean Section	23 (54.8)	178 (57.6)
Prolonged rupture of membrane	25 (59.5)	189 (61.2)
Invasive respiratory support > 1wk	13 (31.0)	133 (43.0)
Hospital stay >3 wk	6 (14.3)	88 (28.5)
Antibiotic exposure >72 h	17 (40.5)	158 (51.1)
Arterial/ venous catheter >72 h	21 (50.0)	168 (54.4)
Total parenteral nutrition	9 (21.4)	71 (23.0)
Persistent thrombocytopenia (<100×10^9L)	19 (45.2)	185 (59.9)

Values in no. (%) or [a]median (IQR). [b]denominators were 19 (in fungal sepsis group) and 148 (without fungal sepsis group). All P≥0.05.

Similar to previous observation, *C. tropicalis* was the predominant fungal pathogen in the current study, followed by *C. albicans* [8]. Sensitivity to antifungal agents was also similar to the previous reports [8,9]. Juyal, *et al.* [9] noted *C. parapsilosis* as the most common species, which might be due to difference in microbiological pattern between tertiary centers. Area under curve of the ROC curve was comparable to previous observations [5,10] and other characteristics of serum BDG were similar to the findings of Mackay, et al. [5] and met the criteria of an excellent screening test [7]. Lower sensitivity and higher specificity was reported by Shabaan, et al. [10], possibly due to use of a higher cut-off value. Variation of BDG between different species of Candida could not be evaluated due to two unidentified isolates. Effect of combining other markers with BDG was also not checked.

Serum BDG level could be used as a diagnostic marker of fungal sepsis in high-risk newborns; however, its use can could only be recommended after further evaluation in multiple settings.

Ethics Clearance: IEC, Burdwan Medical College; No. BMC-B-1058/C, dated July 11, 2017.
Contributors: KI: writing manuscript, collection of data, analysis of data; NK: collection of data, analysis of data, designing study; UM: writing and revising manuscript; KKD: collection of data, analysis of data; KN: planning study, revising manuscript. All authors approved the final version of manuscript.
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