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Diagnostic accuracy of buffy coat culture compared to total blood culture in late-onset sepsis of the newborn☆

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SUMMARY

Objectives: To study the potential of buffy coat culture as a diagnostic tool for neonatal late-onset sepsis.
Methods: This was a study of diagnostic accuracy in newborn infants born at 28–41 weeks of gestation, weighing >800 g, with ≥8 points on the NOSEP-1 scale. Paired samples for total blood culture (TBC) and buffy coat culture were drawn. We established the positivity rate, sensitivity, specificity, predictive values, and likelihood ratios, and compared time to positivity and contamination rates.
Results: Fifty-two newborns were included in the study. Twenty-one TBC and 22 buffy coat cultures were positive. The positivity rate for TBC was 40.4% and for buffy coat culture was 42.3% (p = not significant). Three TBC were positive with negative buffy coat culture. Four buffy coat cultures were positive with negative TBC; Kappa agreement was 0.723, p <0.001. Buffy coat culture sensitivity was 86% (95% confidence interval (CI) 68.5–95.4%), specificity 87% (75.4–93.7%), positive predictive value 82% (65.4–91.1%), negative predictive value 90% (77.9–96.8%), positive likelihood ratio 6.64 (2.79–15.05), and negative likelihood ratio 0.16 (0.05–0.42). We found no difference in time to positivity in hours; Wilcoxon Z = 1224, p = 0.22. The contamination rate was 1.9% for both methods.
Conclusions: Buffy coat culture is as good as TBC for the microbiological diagnosis of late-onset sepsis of the newborn. Buffy coat culture allows the use of remaining plasma for further analysis.

1. Introduction

Neonatal late-onset sepsis (LOS), also known as nosocomial sepsis, occurring after 3 days of age (although some authors consider it after 7 days of age), is a serious problem in neonatal intensive care. The incidence varies between 5% and 32% and depends upon birth weight, gestational age, and the type of germ isolated. It also has a high mortality, with a range of 10–55%, especially in very low birth weight neonates and those infected by Gram-negative organisms or fungi. The most commonly isolated agents are Gram-positive bacteria, and among these, coagulase-negative staphylococci account for most episodes.[1–7] A presumptive diagnosis is made with the presence of signs and symptoms of infection,[8] and confirmation with a positive total blood culture (TBC), which is considered the reference test or gold standard. However, the TBC technique in not without faults, and its diagnostic performance varies, with a sensitivity range of 30–80% and a specificity range of 70–100%.[9–15] Performance is also influenced by the volume of blood used, the culture media, and the manufacturer.[16–21]

Separation and staining of the leukocyte-rich layer of a blood sample, also known as the 'buffy coat',[22,23] which contains mononuclear cells and granulocytes, has been used as an alternative tool for the identification of microorganisms, especially in the diagnosis of tropical diseases.[24] However confidence in its use as a diagnostic tool for sepsis has not yet been attained because of its low sensitivity and specificity.[25–31] We propose the use of buffy coat culture as an alternative to TBC as a new diagnostic tool for LOS in newborns. To our knowledge, there has been no prior attempt to evaluate the diagnostic performance of buffy coat culture for the detection of neonatal LOS compared to the current gold standard, TBC. We hypothesized that the buffy coat culture would perform as well as the reference method (TBC).

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2. Materials and methods

This was a prospective study of the diagnostic accuracy of a new test (buffy coat culture) compared to the reference test (TBC) in neonatal LOS.

2.1. Patients

The study population consisted of all newborns admitted to the neonatal intensive care units (NICUs) of Hospital Materno Infantil de Alta Especialidad and Hospital Metropolitano "Dr. Bernardo Sepúlveda", in the metropolitan area of Monterrey, Nuevo León, Mexico, during the period July 2010 to March 2011. Both hospitals are part of the network of the Secretaría de Salud del Estado de Nuevo León. The study protocol was approved by the research ethics committees of both hospitals and the research ethics committee of Escuela de Medicina y Ciencias de la Salud, Tecnológico de Monterrey. Informed consent was obtained from the parents of all the subjects included.

The selection of patients was done sequentially during morning visits among hospitalized newborns who were suspected for LOS, based on a score of $\geq$8 points on the NOSEP-1 scale;[32] this scale has been validated in our population.[33] Attending physicians did not participate as researchers, but as patient recruiters. The medical researchers were not involved in clinical care decisions. Laboratory personnel remained blinded to the clinical diagnosis of the patients included in the study.

Neonates of both genders were included, born at any gestational ege, who were $\geq$4 days old, and who were being treated in the NICUs of the study hospitals. We excluded patients whose guardians refused to participate and infants weighing less than 800 g, as the extraction of blood for research purposes should not exceed 8% of the estimated blood volume, and at this particular weight the resulting volume would be insufficient for the study and would require an action to replace the blood drawn. Those patients from whom it was not possible to obtain adequate samples were also excluded, as well as those whose samples were damaged during the laboratory process.

2.2. Methods

Paired 2–3-ml blood samples were drawn at the same time and by the same route (peripheral puncture, central line, or percutaneously inserted central catheter). The first sample was used for the reference standard method, TBC, and was inoculated into pediatric blood culture BACTEC PEDS PLUS/F bottles, following the manufacturer's recommendations. The second sample was kept in a sterile tube with ethylenediaminetetraacetic acid (EDTA) (Vacutainer, Cat. No. 367-861) in order to proceed to the buffy coat preparation. This was carried out as follows: the sample was centrifuged at 3500 rpm for 10 min at room temperature in a VanGuard Hamilton Bell V6500 Centrifuge or Mx PowerSpin UNICO centrifuge. This separated the whole blood into an upper layer (plasma), an intermediate layer (rich in leukocytes or buffy coat), and a lower layer (erythrocyte concentrate). Under sterile conditions in a laminar flow hood, plasma was removed with a sterile syringe leaving roughly 1 mm of plasma just above the buffy coat. The buffy coat and residual plasma were slowly aspirated and re-suspended in 0.5 ml of ultrapure water (Gibco Ultrapure distilled water, Cat. No. 10977, Invitrogen) and transferred to a BACTEC PEDS PLUS/F blood culture flask. As expected, some mixing of the buffy coat with the bottom layer of erythrocytes occurred. All samples were processed by one of the authors (JDLV); incubation was done in the laboratory of the hospital at which the blood was drawn.

Cultures were considered contaminated when organisms such as *Propionibacterium acnes*, *Micrococcus* species, viridans group streptococci, *Corynebacterium* species, or *Bacillus* species, as well as when atypical organisms usually unrelated to neonatal infection, were isolated and the patient did not exhibit disease attributable to such serious pathogens. *Staphylococcus epidermidis* was considered a pathogen. The proportion of contaminated cultures in each group was recorded as previously recommended.[34] Each case with a positive TBC was considered as confirmed LOS.

All samples were analyzed in an automated BACTEC 9120 or 9050 (Becton Dickinson Cat. No. 445-800 and 445-570). Cultures were regarded as negative at 7 days if there was no evidence of bacterial growth. The culture media used were those recommended in the literature.[19,35–38] The plates were incubated as recommended: for blood agar, in an Accurate Thermo Scientific or Felisa incubator at 37 °C with 5.5% CO_2, and for MacConkey agar, Columbia agar, and potato dextrose agar, at 37 °C. We quantified the number of microorganisms on each culture plate in accordance with the Kass account, in colony-forming units (CFU) per milliliter. When the growth of two different strains was detected, both were isolated and reseeded on separate culture plates. Inoculums were then prepared to detect the specific strain and its antibiotic susceptibility. A suspension of bacterial colonies taken from the culture plates was used for a turbidity measurement and further analyzed using the VITEK 2 system (bioMérieux, Lombard, IL, USA), as published.[39] Laboratory personnel responsible for reporting the results of the cultures were unaware of the sample preparation technique.

2.3. Statistical analysis

Data were recorded on an Excel 2003 spreadsheet (Office 2003 for Windows, Microsoft Corp. Richmond, VA, USA) and then imported to SPSS v17 (SPSS Inc., Chicago, IL, USA) for analysis. The comparison of diagnostic tests was done with contingency tables, with confidence intervals (CI) at 95%. We compared the time to positivity, measured in hours, and the rate of contaminated cultures. We also measured data regarding diagnostic test performance. As data were not normally distributed, we used the Wilcoxon *Z* test or Kruskal–Wallis test. Cohen's Kappa test was used to evaluate agreement between the tests.

The sample size was calculated as 52 episodes of suspected LOS, with a test power of 80%, contrast ratios of 20%, and a one-sided hypothesis; blood samples had to be taken simultaneously to show that the new method (buffy coat) would be as good as the reference method (TBC). Detecting no difference under these conditions, we could assume that buffy coat culture was as good as TBC in terms of positivity rate, time to positivity, and contamination rates.

3. Results

During the study period, July 2010 to March 2011, there were 13 429 live births at the study hospitals. Of these, 1179 (8.8%) were admitted to the NICUs, and it was from this group that we selected candidates for the study; 54 patients were eligible. Two patients were excluded because the parents refused to participate in the study. The demographic characteristics of the 52 included patients are shown in Table 1.

As can be observed in Figure 1, a Standard for Reporting of Diagnostic Accuracy flow chart,[40] there was no significant difference in the positivity rates of the two methods ($p = 0.48$). Twenty-one of 52 TBC were positive (40.4%), while 22 of 52 buffy coat cultures were positive (42.3%) (p = not significant).

Buffy coat culture sensitivity was 86% (95% confidence interval (CI) 68.5–95.4%), specificity 87% (75.4–93.7%), positive predictive value 82% (65.4–91.1%), negative predictive value 90% (77.9–96.8%),