

The usefulness of the buffy coat smear and panbacterial polymerase chain reaction in early diagnosis of neonatal sepsis

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ABSTRACT

Objective. In this study are evaluated the usefulness of the buffy coat smear and panbacterial polymerase chain reaction (PCR) as diagnostic tests in the early detection of neonatal sepsis. Material and methods. It was studied 49 patients aged up to 28 days who were hospitalized in the Intensive Care Unit (ICUs) of the Neonatology, with a clinical diagnosis of neonatal sepsis and 49 umbilical cord samples of healthy newborns. Blood cultures and 50 μ L of plasma were taken for the DNA and performance of the broad-range PCR primer system (panbacterial PCR). Simultaneously, were taken three capillaries with blood for the leukocyte layer (buffy coat) smear, we performed three stains: Gram; Löeffler blue methylene (LBM), and acridine orange (AO). Statistical analysis included sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) against the clinical diagnosis. Results. With respect to stains of buffy coat smear, they resulted very specific, from 90-97%, with 64-75% sensitivity, 87-94% PPV, and 77-82% NPV. In inverse fashion, PCR resulted very sensitive at 96%, with 91% specificity, 92% PPV, and 96% NPV. Conclusions. Buffy coat smear stains are easy, fast, and specific, while that of PCR was highly sensitive. Thus, both can be utilized as diagnostic tests.

Key words. Bacteriologic stains. Molecular diagnosis. Neonatal sepsis. Buffy coat.

Utilidad de la tinción de buffy coat y reacción en cadena de la polimerasa panbacteriana en el diagnóstico temprano de sepsis neonatal

RESUMEN

Introducción. En este estudio se evaluaron la utilidad del frotis de la buffy coat y la reacción en cadena de la polimerasa (PCR) panbacteriana, como pruebas de diagnóstico para la detección temprana de la sepsis neonatal. Material y métodos. Se estudiaron 49 pacientes de hasta 28 días de edad, hospitalizados en la Unidad de Cuidados Intensivos Neonatales con diagnóstico clínico de sepsis neonatal, y 49 muestras de cordón umbilical de recién nacidos sanos. Se tomaron hemocultivos y 50 μL de plasma para la extracción del ADN y un sistema de iniciadores para PCR de amplio rango (PCR panbacteriana). Al mismo tiempo se tomaron tres capilares de sangre para el frotis de la capa de leucocitos (buffy coat), después se realizaron tres tinciones: Gram, azul de metileno de Löeffler y naranja de acridina. El análisis estadístico incluyó la sensibilidad, especificidad, valor predictivo positivo (VPP) y valor predictivo negativo (VPN) en contra del diagnóstico clínico. Resultados. Las tinciones de los frotis de buffy coat resultaron muy específicas, de 90-97%, con 64-75% de sensibilidad, 87-94% de VPP y 77-82% de VPN. De manera inversa, PCR resultó muy sensible en 96%, con 91% de especificidad, 92% de VPP y 96% de VPN. Conclusiones. Las tinciones de buffy coat son fáciles, rápidas y específicas, mientras que PCR fue sumamente sensible. Por lo tanto, ambas pueden utilizarse como pruebas diagnósticas

Palabras clave. Tinciones bacteriológicas. Diagnóstico molecular. Sepsis neonatal. Buffy coat.

INTRODUCTION

Neonatal sepsis is defined as the systemic inflammatory response syndrome arising from an infectious process that presents during the first 28 days of extrauterine life. It is considered as a health problem worldwide due to its high indices of morbility and mortality. In Mexico, there are between 4 and 15 cases per 1,000 live births; in Intensive Care Units (ICUs), this amount is 5-20 times higher.

To establish an adequate diagnosis of sepsis, we must evaluate clinical and laboratory aspects. In a previous study conducted by our work group, we decided to evaluate 11 indices of infection in global fashion (high C reactive protein, increased erythrocyte sedimentation rate (ESR), leukopenia, neutropenia, bandemia, increased band/neutrophils, plaquetopenia, anemia, inmadure/neutrophil ratio, fibrinogen and haptoglobin) arbitrarily assigning one point for each test alteration observed. In patients with > 5 positive indices, sensitivity and sensitivity were found near equilibrium (71.4 and 68%, respectively) with 69% efficiency in the clinical test. The blood culture is the microbiological gold standard, but its sensitivity ranges between 20 and 80%.

It has been reported in some articles⁷⁻⁹ that by means of supernatant study of the centrifuged blood capillary, different stains can be carried out for detection of bacteria, and even of fungi and yeasts; this test is called the buffy coat smear.

In addition to traditional methods for identifying microbial presence, it has been demonstrated that recognition of DNA bacterial sequences possesses panspecific advantages, such as those associated with the case of the gene $16S\ rRNA$ through the polymerase chain reaction (PCR)^{10,11} and real-time PCR.¹²

Diagnosis of sepsis, is clinical and based on culture results. Although blood culture is the gold standard for the diagnosis, culture reports would be available only 48-72 h, and have low sensitivity. The earliest signs are often subtle and nonspecific, these signs include changes in respiration, apnea and/or tachypnea; in heart rate, including frequent episodes of bradycardia and/or tachycardia; in ventilation and cardiac rate. In addition, neonates with sepsis commonly exhibit alterations in core body temperature, as manifested by fever and or hypothermia. Changes in skin perfusion commonly accompany sepsis, as manifested by mottling, cooling of the extremities, and a general "ill look" to the baby overall. Other subtle findings of sepsis include the development of feeding intolerance, vomiting, or diarrhea.

Accurate and timely diagnosis of early onset neonatal sepsis remains challenging to the clinician and the laboratory. Many potential markers (acute phase reactants, cell surface markers, cytokines) are not routinely available to the laboratory. Most tests are measured on a continuous scale where there is overlap between infected and non-infected infants. Total leukocyte count, immature to total neutrophil ratio, platelet count, and CRP (C reactive protein), have low sensitivity and specificity or varying delayed responses early in the course of infection. Leukocyte indices and CRP are considered to be "late" markers and are not sensitive enough for early diagnosis of neonatal sepsis.

OBJECTIVE

Of the study was to evaluate the usefulness of Gram, acridine orange, and Löeffler blue methylene stainings of the buffy coat smear and of the panbacterial PCR as early diagnostic tests for neonatal sepsis.

MATERIAL AND METHODS

We studied 49 neonatal patients aged up to 28 days who were hospitalized in the Intensive Care Unit (ICUs) of the Neonatology Service Hospital General Dr. Gaudencio González Garza from August 2005 to July 2006 with a clinical diagnosis of neonatal sepsis without antibiotic treatment only in 4% or with a maximum of a 48-h antibiotic treatment, or even with > 3 days without a clinical response, since the hospital is a Tertiary medical care. We used various combinations of antibiotics, the scheme used was ampicillin/amikacin, second cefotaxime/vancomycin and to a lesser extent cefotaxime/amikacin. We performed a blinded aleatorized study. As the control group, 49 blood samples in which the absence of pathogens was confirmed by blood culture, were provided by the Umbilical Cord Pluripotent Hematopoietic Cell Bank that pertains to the Central Blood Bank of the Mexican Institute of Social Security Institute's (IMSS's) La Raza National Medical Center (BSC-CMNR). The protocol was accepted by the local Research and Ethics Committee of the Hospital, and all parents or guardians signed the letter of informed consent.

Sample calculation

We calculated the sample size through the program: Interference for proportions: Comparison of 2

independent samples. Considering P1 (proportion of the population 1) and the detection rate of the "buffy coat" which is 75 % (8) and P2 (the proportion of the population 2) and the detection rate of panbacterial PCR in vitreus is 95%, ¹⁰ with a value of alpha equal 0.05 for an expected proportion of 80%, giving a total of 49 diseased patients and 49 new born without disease. This program is based on the normal approximation to the binomial distribution.

Clinical diagnosis of neonatal sepsis

Alteration of rates of infection at least 5 (leukocytosis or leucopenia, neutrophilia or neutropenia, thrombocytopenia, presence of bands, presence of vacuolated neutrophils or toxic granulations, bands/total neutrophils ratio, immature/mature ratio altered, hemoglobin, ESR, CRP increased and positive blood culture. The diagnosis of neonatal sepsis based on a number of risk factors and nonspecific clinical and laboratory paramentes, there is no validated scale. Aproximately, sepsis screen *per se* had a sensitivity and negative predictive value (NPV) of 48.3 and 78.3%.

Inclusion criteria

Newborn patients with changes in respiration, heart rate, ventilation and cardiac rate. Alterations in core body temperature, changes in skin perfusion, cooling of the extremities, and a general "ill look" to the baby overall. The development of feeding intolerance, vomiting, or diarrhea. In addition, we evaluated 11 indices of infection in global fashion (high C reactive protein, increased ESR y, leukopenia, neutropenia, bandemia, increased band/neutrophils, plaquetopenia, anemia, inmadure/neutrophil ratio, fibrinogen and haptoglobin) arbitrarily assigning one point for each test alteration observed. In patients with > 5 positive indices, sensitivity and sensitivity were found near equilibrium.

We performed the following on patients under aseptic conditions: venous puncture or catheter aspirate, obtaining approximately 500 μL of blood for buffy coat smear and PCR. Additionally, the remainder of the laboratory tests was conducted, taking samples for complete blood work, blood culture (BacT/Alert®), C-reactive protein, lumbar puncture for cytochemical study and culture, uroculture, and umbilical cord culture. Procedures for these tests were carried out in the Hospital's laboratories by authorized personnel with conventional techniques. The control group was made buffy coat smear and

PCR. The gold standard was the clinical diagnosis, which includes: clinical parameters, laboratory and blood culture.

DNA extraction

Care was always taken during and after obtaining patient samples to prevent extraneous environmental contamination. For example, the skin surface was prepared by swabbing with alcohol prior to obtaining the sample; samples were immediately placed in sterile microfuge tubes. The DNA was isolated with guanidine isothiocyanate and phenol utilizing 500 $\mu \rm L$ of TRIzol reagent (Gibco BRL) according to the procedure described by Chomczynski. 13 The DNA was resuspended in 50 $\mu \rm L$ of distilled water after precipitation with ethanol at 75%. This solution was heated at 55 $^{\rm o}{\rm C}$ for 20 min. We determined its absorbance relationship at 260/280 nm.

Panbacterial PCR

The broad-range PCR primer system has been used previously to screen synovial DNA preparations. ¹⁰ Briefly, this is a non-nested assay system that employs two primers:

- 5'-GCGTTAATCGGAATTACTGGGCGTAAG-3', and
- 5'-GGTTGCGCTCGTTGCGGGACTTAACC-3'.

Cycling parameters were 4 min at 95 °C, then 35 cycles for 1 min at 95 °C, I min at 52 °C, 1 min at 72 °C, and finally 10 min at 72 °C. This primer system is referred to as panbacterial, because it amplifies 16S ribosomal RNA (rRNA) gene sequences from a number of bacterial species. As designed, the system amplifies a DNA fragment of \sim 577 bp (depending on the organism), 10 spanning the region from nucleotides 501-1,077 in the standard (*Escherichia coli*) 16S rRNA gene.

As with other similar systems, the rationale for the design of this broad-range primer system resides in the observation that prokaryotic 16S rRNA gene sequences have been conserved over evolution, and that these sequences have been especially well conserved in particular regions that function importantly in the ribosome. ¹⁴ Design of consensus primers based on these highly conserved DNA sequence regions allows amplification of rDNA coding sequences from many, although not all, bacterial rRNA 16S genes, determined by a homology search of primer sequences in GenBank as follows: Bacillus;

Borrelia; Campylobacter; Escherichia; Listeria; Neisseria; Proteus; Rickettsia; Salmonella; Staphylococcus; Yersinia; Bordetella; Chlamydia; Klebsiella; Mycoplasma; Pseudomonas; Shigella; Serratia; Streptococcus, and others. Sensitivity generally lies within the detection range of 10-50 bacterial cells.

For each assay run, water controls (i.e., no added template) were interspersed among the samples to be analyzed. Pure DNA from *E. coli* was utilized as a positive control for the panbacterial screening system. Amplification products were analyzed on ethidium bromide-stained agarose gels. The tests were performed in duplicate.

Buffy coat smear

We mounted three capillaries for each patient and control with blood samples in sterile fashion and fixed them with heat. These were centrifuged at 3,000 rpm for 15 min to obtain three layers in the capillary; the plasma; the leukocyte layer or "buffy coat", and the erythrocyte layer. We cut the capillary with a diamond-point marker at the height of the erythrocyte layer and also at that of the plasma, slightly above the buffy coat, and emptied the contents into three clean, degreased, and sterile containers. The drops of buffy coat were resuspended in PBS, and we proceeded to carry out the smears. The latter were first allow to air dry and then methanoldried for 2 min.

For each patient and control, we performed three stains: Gram; Löeffler blue methylene (LBM), and acridine orange (AO). Observation by microscope was conducted for a minimum of 15 min per plate prior to being determined as negative. The tests were performed in duplicate.

RESULTS

Within the study group of patients, 68% were males, while 32% were females; on the other hand, in the control group there was gender equity. The percentage of mortality was 18.9% in patients with sepsis.

The risk factors most frequently found in the group of patients with a clinical diagnosis of sepsis were the following: parenteral nutrition, (100%); catherer use, (97%); prolonged fasting, (92%); mechanical ventilation, (89%); cesarean birth (67%); malnutrition (57%); low birth weight, (38%); premature rupture of membranes, (31%), and surgical procedures (27%). There were no associated risk factors with regard to the control group.

In the hematologic and serum examinations performed, we established the following changes in several parameters, (Table 1). In addition, there was development in the cultures performed to identify probable sources of infection: 33% in urocultures; 27% in blood cultures; 15% in cerebrospinal fluid (CSF) culture; 15% in catheters, and 3% in umbilical cord secretion. We used various combinations of antibiotics, the scheme used was ampicillin/amikacin, second cefotaxime/vancomycin and to a lesser extent cefotaxime/amikacin. There were no positive cultures in the control group.

We found 33 cases (67%) (Table 2) in the distinct cultures carried out that resulted positive, but the actual number of patients whit multiple infections demonstrated by culture test was 29 (59%).

We also analyzed all of the cultures together to determine which microorganisms predominated as causes of infection. Among the results, it was noteworthy that CNS (coagulase-negative *Staphylococcus*) was the cause of 44.8% of the infections, followed by *E. coli*, the cause of 31%. We also found *Candida sp.* and *Klebsiella* in 10.8% each, *Pseudomonas sp.* and *Enterobacter sp.* in 5.4% each, and *Enterobacter cloacae* in 2.7%.

With respect to bacteria found in the buffy coat smear, we found bacterial forms in 39 patients (80%), in which extracellular Gram-positive cocci predominated in 15 of the patients; of these, three died. We also observed extra- and intracellular Gram-positive cocci (phagocyted by polymorphonuclears) in seven patients; of these, three died. Similarly, we found 14 cases with Gram-negative

Table 1. Neonatal data, clinical signs and laboratory parameters.

Data	Media (rank)		
Gestational age (weeks)	34.2 (27-40)		
Birthweigth (grams)	2,030 (915-3,500)		
Age at onset of sepsis (days)	14.8 (2-27)		
Clinical signs			
Dysthermia	95%		
Respiratory alterations	54-0.5%		
Laboratory parameters			
Neutrophilia	76%		
Vacuolated neutrophils/toxic granulations	46%		
B/N ratio	38%		
Acid-base disequilibrium	70%		
*CRP-value (mg/dL)	5.8% (0-25.2)		

*CRP: C reactive protein, cut-off value for "positive", 0.31 mg/dL. B/N: band/neutrophil.

bacilli and three cases with Gram-positive bacilli (Table 3).

In the smears carried out in control-group patients without sepsis, we found bacteria in four cases (8%), observing in two cases extracellular Gram-positive cocci and in the remaining two, Gram-positive bacilli.

With regard to the number of patients with a clinical diagnosis of sepsis, performed by physician and detected by panbacterial PCR, we found that in the

Table 2. Isolated microorganisms in cultures of the study group.

Culture type (Number of cultures in patients with sepsis)	Isolated microorganisms
Uroculture (13)	 4 E. coli 2 Enterobacter sp. 2 E. coli/Candida sp. 1 E. coli/Pseudomonas sp. 1 Pseudomonas sp. 1 Coagulase-negative Staphylococcus 1 Coagulase-negative Staphylococcus/Candida 1 Candida sp.
Blood culture (9)	8 Coagulase-negative <i>Staphylococcus</i> 1 <i>Enterobacter cloacae</i>
CSF (5)	4 Klebsiella spp. 1 Coagulase-negative Staphylococcus
Catheter (5)	2 Coagulase-negative Staphylococcus1 Staphylococcus aureus1 E. coli1 Candida sp.
Umbilical secretion (1)	1 E. coli
Total	33

E. coli: Escherechia coli. CSF: cerebrospinal fluid.

Table 3. Microorganisms observed in buffy coat smear stains.

group of patients, we were able to appreciate a 577-bp band in 47 of cases (96%), while in the control group, we only observed this in four cases (8%).

In table 4, we grouped patients taking into account the microbial group identified and compared the latter with the results obtained from the stains and the panbacterial PCR, because these are interesting for understanding the importance of the application of our methodologies. In the remaining positive cultures, we did not observe agreement with our data.

With all of these results, we calculated percentages of sensitivity, specificity, Positive predictive value (PPV), and NPV for each of the stains and for PCR with traditional formulae according to 2 x 2 contingency tables. ¹⁵ We considered that the gold standard for each of these was the clinical diagnosis (> 5 changed parameters), in this respect, we determined the usefulness with regard to confirmation of the clinical suspicion, diagnostic support, treatment restoration in the case of a positive test, and compatibility with the remainder of laboratory and medical office examinations (Table 5).

DISCUSSION

We observed that in the group of patients, there was a predominance of more than double of masculine over feminine gender (68 and 32%, respectively). This tendency is cited by other authors.^{3,16}

As in the majority of cases reported, the clinical signs and symptoms observed were characterized by their variety and unspecificity. The main clinical manifestations found were dysthermia in 95% of cases, coinciding with the findings of other authors, and acid-base disequilibrium in 70%.

As we mentioned previously, to carry out a correct early diagnosis, we require the support of several laboratory data; a sole datum does not support the diagnosis. In this study, the changed parameters that stood out were, in first place, C-reactive

Bacteria type	Patients		Controls	
	Positive cases (n = 39)	Mortality in positive cases	Positive cases (n = 4)	Mortality in positive cases
Extracellular Gram-positive cocci	15	3	2	0
Extra- and intracellular Gram- positive cocci	7	3	0	0
Gram-negative bacilli	14	0	0	0
Gram-positive bacilli	3	0	2	0

Table 4. Comparison of polymerase chain reaction (PCR) and buffy coat smear results with different positive cultures.

Number of patients (%)	Number of deaths (%)	Blood culture	Related positive cultures	PCR	Buffy coat smear	
Patients with clinic	cal diagnosis of sepsi	S				
5 (10)	3 (6)	Coagulase-negative Staphylococcus	1 catheter (same)	+	Extracellular Gram-positive	
2 (4)	0	Coagulase-negative Staphylococcus	1 catheter (same)	+	<u>-</u>	
1 (2)	0	Coagulase-negative Staphylococcus	0	+	-	
1 (2)	0	Enterobacter cloacae	0	+	Gram-negative bacilli -	
10 (21)	0	-	0	+	Extracellular Gram-positive cocci +	
7 (14)	3 (6)	-	1 catheter Staphylococcus aureus	+	Extra- and intracellular Gram-positive cocci	
13 (27)	13 (27) 0 -		4 CSF <i>Klebsiella sp.</i> urocultures + 5 <i>E. Coli</i> 2 <i>Enterobacter sp.</i> 2 <i>Pseudomonas sp.</i>		Gram-negative bacilli -	
2 (4)	0	-	0	+	Gram-positive bacilli +	
6 (12)	1 (2)	-	0	+	· -	
1 (2)	Ò	-	0	-	Gram-positive bacilli +	
1 (2)	0	-	0	_	· -	
Total = 49 (100	0%)					
• Controls						
45 (92%)	0	-	0	-	-	
2 (4%)	0	-	0	+	Gram-positive bacilli	
2 (4%)	0	-	0	+	Gram-positive cocci	
Total = 49 (100	0%)				·	

CSF: cerebrospinal fluid. E. Coli: Escherichia coli.

Table 5. Statistical values obtained from buffy coat smear stains and from panbacterial polymerase chain reaction (PCR) compared with the clinical diagnosis.

Clinical diagnosis					
	Panbacterial PCR (%)	Gram stain (%)	Staining with AO (%)	Staining with LBM (%)	
Sensitivity	96	72	75	64	
Specificity	91	95	90	97	
Positive predictive value (PPV)	92	94	87	94	
Negative predictive value (NPV)	96	77	82	82	
Precision	94	84	84	85	

AO: acridine orange. LBM: Löeffler blue methylene.

protein, found altered in 84 %, on which there are data that this is a highly sensitive, but not a highly specific, marker,⁵ in that it is changed in many inflammatory processes, thus causing false-positives for sepsis. In second place, we found total amount of neutrophils, altered in 76%, according to Manroe's reference ranges for neutrophils indices.¹⁸

In the blood cultures, there was low recuperation of microorganisms (only 18%), which was worrisome. One of the causes for this may be high dilution in the blood culture due to the small volumes of blood. Normally, a relationship of blood volume:mean of 1:10 is recommended; ¹⁹ also recommended is that these tests be carried out at a minimum of twice to increase possibility of detection. ²⁰ This was very difficult to perform with the majority of patients, due to their weight, associated pathologies, etc. Other causes can be the transitory or intermittent nature of the bacteremia, the possible low density of the bacteria present, or even an inhibitory effect of the antibiotic therapy *intra-* or *extrapartum*. ²¹

The main microorganism isolated was coagulasenegative Staphylococcus, 89% of positive blood cultures and 35% of the remaining cultures performed. The finding of CNS as the outstanding agent in blood cultures coincides especially with that published in previous studies conducted by our work group^{2,5} and in Mexico in general,^{3,21} where it is observed that these are isolated from approximately 46-57% of positive blood cultures. It is noteworthy that in all patients with blood culture positive to coagulase-negative *Staphylococcus*, the disease was detected after the third day of life, that is, the former can be considered as cases of late-origin or nosocomial sepsis, while in the case of the patient with a blood culture positive for E. cloacae, this was determined on the first day of life. Thus, we can think that this was early sepsis. As can be observed in table 2, another of the principal microorganisms isolated from the distinct cultures was E. (approximately 30% of the isolations). This coincides with that reported by other authors in Mexico. 16

About the matter to the diagnosis of neonatal sepsis, our work team previously reported that, the smear of the buffy coat stained with Gram had a good specificity, taking as gold standard the blood culture. We decided to investigate what type of stain was the most appropriate for detecting bacteria in blood. The Gram stain was not ideal for staining blood; however, several authors utilize this stain because it is essential for classifying bacteria such as Gram-positive or -negative and to renovate the antibiotic picture. In addition, blue methylene

and acridine orange stains have been employed with excellent results observed. We found that Gram-positive cocci predominated over Gram-negative bacilli, as can be observed in table 3. This is to a certain degree contrary to what we expected, because it was cited that neonatal sepsis is caused mainly by Gram-negative bacilli, especially enterobacterias (between 40 and 60%); among these, the mortality rate ranges between 30 and 90%.^{3,23} However, this did not occur in this study. The highest percentage of death was related with the finding of Gram-positive cocci, (14%) and none with Gram-negative bacilli. We also found Gram-positive bacilli in the study group; it is probable that this was *Listeria mono*cytogenes, in that the latter is the most commonly found cause of sepsis in neonates.¹⁷

It is important to note that in control group, we found four cases of samples with bacteria, two with Gram-positive cocci and two with Gram-negative bacilli, which can be due to various causes: auto-contamination from the sample-taking stain, to inadequate asepsis at site of the puncture or material contamination, false-positive on observing it, or transitory bacteremia, because the blood culture was negative. Among the findings of Gram-positive cocci in patients, we discovered two variants: extracellular Gram-positive cocci in 15 patients, and intracellular Gram-positive cocci in seven (phagocyted in polymorphonuclears). Of these, we establish the distinction that despite that there is the same amount of deaths in both cases, we noted that there was a tendency toward greater mortality in the case of intracellular cocci. Contrary to what would be thought if bacteria were observed outside of the leukocytes, which could reflect a poor response or deficient immune mechanisms (poor opsonization),⁸ what is certain is that leukocytes infected in peripheral blood usually indicate a disease with a fatal result.²⁴ Thus, it has been considered as entertaining a poor prognosis. It is thought that the genus Staphylococcus especially can utilize the phagocyte as a "Trojan horse" and be transported at a distance, which sometimes can be the origin of new septic foci.²⁵ Studies on bacteremia in rabbits suggest that phagocyted Staphylococcus pathogens are free from damage at the intracellular location, because they derive from a microambiance that is protected from destruction by the immune system of the host, and in this manner they multiply. 7,25

By the way, the levels of adhesion molecules had been detected in sepsis, sICAM-1 (soluble intercellular adhesion molecule) and sE-selectin (soluble endothelial selectin), these levels were related to severe sepsis. Both tests have the advantage that seem to be good and early predictors of the beginning of sepsis, and also they seem to have a prognostic value for the severity, but they are expensive and inaccessible.²⁶ Also, it was described a novel manual method that included use of a serum separator tube to early diagnostic of sepsis, and it is cost-effective.²⁷ It is important to observe the low recuperation of microorganisms in blood cultures on comparing these with the positive results of PCR as well as of the buffy coat smear, thus exhibiting the importance of their application. Also of great value was positive PCR by itself due to its high sensitivity, in that among six cases in which one patient died, the blood culture, other cultures, and the buffy coat smear resulted negative. One aspect that commands our attention was the case of the patient with a diagnosis of sepsis, but with negative cultures, PCR, and buffy coat smear. Perhaps this was a microorganism of difficult detection, for which a search is not commonly in the laboratory, for example, *Ureaplasma sp.*

Panbacterial PCR was able to discard for us a great percentage of patients those who were and who were not infected. This type of PCR only detects bacterial-origin sepsis; therefore, it would be recommended to identify the class and morphology of the bacteria with Gram staining of the buffy coat smear.

The five cases with three deaths attract our attention; in these, we found CNS in the blood culture, in agreement with the PCR and the buffy coat smear staining; in addition, there was a catheter culture that coincided with the blood culture. After this we had two patients with CNS-positive blood cultures, positive PCR, but negative buffy coat smear, comprising two examples of false-negatives for the buffy coat smear; perhaps the amount of bacteria in the blood was not sufficient for detection on the slide. There were also seven patients, of whom three died. In the seven, we detected extra- and intracellular Gram-positive cocci, but the blood culture did not develop; this can be due to that the bacteria are within the leukocytes, diminishing in this manner the number of circulating bacteria and with this, the possibility of recuperating these in the culture.

In synthesis, in order to confirm or discard the clinical suspicion of infection, buffy coat smear staining is cheap, it can be applied to routine laboratory, is very specific, but its sensitivity is low, the panbacterial PCR, is expensive, but its sensitivity is high, both methods were useful, although the performance of the blood culture is necessary for antimicrobial sensitivity. The staining has the limiting

that depends on the expertise of the analyst and the laboratories implement it must have good systems of control and quality assurance including the analysis of panels submitted by an outside institution. According to the clinical data, sepsis arising from nosocomial-source bacteria predominated.

It is important to possess knowledge on the microorganisms present in a hospital service or institution, as well as the sensitivity *in vitro* of the antibiotics employed empirically, because from these we are able to establish adequate programs of prevention and to select early antimicrobial schemes that can improve survival in neonates who acquire an intrahospital infection.

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ABBREVIATIONS

- **CRP:** C reactive protein.
- **B**/**N**: band/neutrophil.
- E. coli: Escherechia coli.
- **CSF:** cerebrospinal fluid.
- AO: acridine orange.
- **BM:** Löeffler blue methylene.

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