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The profile of ancillary laboratory tests in neonates with bacterial and/ or fungal sepsis

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THE PROFILE OF ANCILLARY LABORATORY TESTS IN NEONATES WITH BACTERIAL AND/ OR FUNGAL SEPSIS

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A dissertation submitted to the faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Masters in Medicine.

Johannesburg 2020

DECLARATION

I, Lino Sono, declare that this research report is my own work. It is being submitted for the Degree of Masters of Medicine in the branch of Paediatrics, in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signed: _____

On this: _____ day of: _____, 2020

DEDICATION

My greatest treasure is my family. Thank you for being my rock and anchor even when I faltered. My family is my favourite. My God bless you abundantly.

This is also dedicated to all my friends who prayed for me and were a voice of hope and encouragement throughout.

PUBLICATION AND PRESENTATIONS ARISING FROM THE DISSERTATION

1. United South African Neonatal Association Conference; Port Elizabeth, 12-15 September 2019
2. The University of the Witwatersrand, Faculty of Health Sciences, School of Medicine Biennial Research day 23 October 2019

ABSTRACT

Background: Blood and cerebrospinal fluid (CSF) cultures are used as the gold standard to diagnose neonatal sepsis/meningitis. A challenge in their use is low yield, and limited availability, especially in low-resource settings. We evaluated profiles of complete blood count (CBC), c-reactive protein (CRP), and CSF cell count and protein in neonates with culture-proven sepsis/meningitis.

Methods: Neonates with positive blood and/or CSF cultures who had results of CBC, CRP, CSF cell count, and protein performed within 24-48 hours of culture were enrolled. The proportion of neonates with abnormalities in these tests was determined and comparisons among different types of pathogens were performed.

Results: A total of 942 isolates were cultured in blood and/or CSF. Groups of organisms isolated were Gram-negatives (GN) (62.0%), Gram-positives (GP) (23.4%), and Candida (14.6%). Common abnormality in CBC was thrombocytopenia, observed in 30% of neonates with culture-proven sepsis. There was a higher proportion of thrombocytopenia among GN- (39.9%) and Candida-infected (44.6%) compared GP (15.1%)($p<0.001$). Leukopenia was relatively more common among GN than GP (20.8% vs 8.4%; $p < 0.001$). Seventy percent had high CRP (≥10mg/L). Only 26.7% and 61.6% had abnormal CSF cell count (>20cells/mm^3) and high protein (>150mg/dL) among those with positive CSF cultures.

Conclusion: The majority of neonates with positive blood or CSF cultures have normal CBC or CSF cell count respectively, therefore the absence of abnormalities in these parameters cannot be used solely to exclude sepsis. CRP appears to be the most useful test in diagnosing sepsis as it is abnormal in 70% of patients with culture-proven sepsis.

ACKNOWLEDGEMENT

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ABBREVIATIONS

CBC- Complete blood count
CHBAH - Chris Hani Baragwanath Academic Hospital
CP - Culture proven
CP-NSM - Culture proven neonatal sepsis or meningitis
CRP- C-reactive protein
CSF- Cerebrospinal fluid
HREC- Human Research Ethics Committee
I/T ratio - Immature to total neutrophil ratio
ITNR- Immature to total neutrophil ratio
NHLS - National Health Laboratory Services
NSM – Neonatal sepsis or meningitis
WBC-Diff - White blood cell differential count
WCC - White cell count

PLAGIARISM DECLARATION

I, Dr. Lino Sono (Student number: 9301635V) am a student registered for the degree of Masters in Medicine in Paediatrics in the Academic year 2019.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
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- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
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RESEARCH REPORT IN PUBLICATION SUBMISSIBLE FORMAT

INTRODUCTION

Sepsis has been described as a systemic inflammatory response syndrome (SIRS) to pathogens such as bacteria, viruses, or fungi that is associated with haemodynamic changes and other clinical manifestations (1-3). The definition of sepsis also includes isolation of pathogens from normally sterile body fluids such as blood and cerebrospinal fluid (CSF). Blood and/ or CSF culture is the gold standard in the diagnosis of sepsis. In resource-limited settings, healthcare facilities might not have access to blood culture results for days after working up a patient with possible serious bacterial infection (pSBI). Waiting for culture results will therefore result in delays in initiating antibiotics in neonates with pSBI. This often results in patients being treated based on clinical signs suggestive of sepsis and available laboratory ancillary tests, namely complete blood count (CBC) and/or c-reactive protein, (CRP) whose results are relatively more readily available than blood cultures. Even when blood cultures are accessible, they are only available after 24 hours and the yield is low despite a patient having clinical signs suggestive of sepsis.

Delayed initiation of appropriate treatment for sepsis is associated with increased morbidity and mortality (4, 5), thus antibiotics are started or continued based on abnormalities in CBC, white blood cell differential count (WBC-Diff), and CRP. The proportion of patients with bacterial or fungal culture-proven sepsis who have abnormal ancillary laboratory tests is not well known, especially in settings where there is a high incidence of sepsis. Knowing these proportions will assist attending physicians in estimating the likelihood of patients having sepsis due to bacteria or fungal infection in settings where culture is negative or not available. In this study, we sought to determine abnormalities in CBC, WBC-Diff, and CRP in neonates with bacterial or fungal organisms considered pathogens isolated from blood; and to determine cerebrospinal fluid (CSF) cell count and protein abnormalities in neonates with organisms isolated in CSF.

PATIENTS AND STUDY METHODS

This was a retrospective study conducted at Chris Hani Baragwanath Academic Hospital (CHBAH), a public tertiary hospital in Soweto, South Africa. The study population were neonates admitted to the neonatal unit at CHBAH suspected of having sepsis during their admission and identified to have a positive blood and/or cerebrospinal fluid (CSF) culture from January to December 2017. Potential participants were identified by searching the

NHLS database for neonates with positive blood and CSF cultures during the period of the study. They were included if one or more ancillary tests were available. Where 2 or more organisms were cultured from a single patient these were considered as separate infections if the interval between cultures was more than 48 hours at least since CRP was allowed up to 48 hours later. *Corynebacterium*, *Bacillus,* and coagulase-negative *Staphylococcus* were considered as contaminants and thus were excluded from the analysis. Ancillary laboratory tests assessed were total white blood cell count (WBC), platelet count, WBC-Diff, CRP levels, CSF cell count, and protein. The CBCs that were assessed were only those that were done within 24 hours of culture and for CRPs it was only those that were done within 48 hours of culture.

Definitions of abnormalities for different parameters on laboratory ancillary tests were as follows; leucocytosis- white blood cell count greater than 25 x 10^9/L, leukopenia- white blood cell count less than 5 x 10^9/L, thrombocytopenia- platelet count less than 100 x 10^9/L, neutrophilia- neutrophil count greater than 7 x10^9/L, neutropenia- neutrophil count less than 1.75 x10^9/L, increased immature to total neutrophil ratio- ratio equal and greater than 0.2, high CRP- CRP equal and/or greater than 10 mg/L, abnormal CSF white cell count- cell count greater than 20 cells/mm³ and high CSF protein- protein greater than 150 mg/dL.
The data was captured into an Excel spreadsheet and analysed using Statistica. Comparisons in the proportion of patients with abnormalities in blood or CSF parameters among the different organisms were made using chi-square. The difference with a p-value < 0.05 was considered statistically significant. The study was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand.

RESULTS

Organisms isolated from sterile sites

There were 942 organisms considered pathogens that were isolated from sterile sites of neonates with pSBI over a 12 month period, 886 in blood and 106 in CSF (Table 1). Gram negatives were the most common type of organisms isolated in both blood and CSF (n = 584; 62.0 %). The most common bacteria isolated among the Gram-negatives were *Acinetobacter baumannii* (n = 271; 46.4%) and *Klebsiella pneumoniae* (n = 198; 33.9%). Among the Gram-positives the common bacteria were *Enterococcus* species (n = 74; 33.6%), *Staphylococcus aureus* (n = 47; 21.4%) and *Streptococcus viridans* (n = 46; 20.9%) while among the Fungi the common fungi were *Candida parapsilosis* (n = 86; 62.3%) and *Candida albicans* (n = 38; 27.5%).

Table 1 Pathogens isolated from blood and/or cerebrospinal fluid

	Blood or CSF N = 942	Blood N = 886	CSF N = 106
Gram Negatives	**584 (62.0)**	**543 (61.3)**	**84 (79.2)**
Acinetobacter baumannii	271 (46.4)	247 (45.5)	54 (64.3)
Klebsiella pneumonia	198 (33.9)	191 (35.2)	20 (23.8)
Pseudomonas species	32 (5.48)	30 (5.52)	2 (2.38)
Escherichiae coli	29 (4.97)	27 (4.97)	2 (2.38)
Enterobacter species	20 (3.42)	17 (3.13)	3 (3.57)
Klebsiella oxytoca	6 (1.03)	6 (1.10)	0 (0)
Achromobacter xylosoxidans	4 (0.68)	4 (0.74)	0 (0)
Providentia species	4 (0.68)	4 (0.74)	0 (0)
Neisseria species	3 (0.51)	3 (0.55)	0 (0)
Acinetobacter lwoffi	2 (0.34)	2 (0.37)	0 (0)
Others	15 (2.57)	12 (2.21)	3 (3.57)
Gram Positives	**220 (23.4)**	**205 (23.1)**	**18 (17.0)**
Staphylococcus aureus	47 (21.4)	46 (22.4)	2 (11.1)
Streptococcus viridans	46 (20.9)	41 (20.0)	5 (27.8)
Enterococcus faecium	41 (18.6)	36 (17.6)	7 (38.9)
Enterococcus feacalis	33 (15.0)	31 (15.1)	2 (11.1)
Listeria monocytogenes	26 (11.8)	25 (12.2)	1 (5.56)
Streptococcus agalactiae	23 (10.5)	22 (10.7)	1 (5.56)
Others	4 (1.82)	4 (1.95)	0 (0)
Candida and other yeasts	**138 (14.6)**	**138 (15.6)**	**4 (3.78)**
Candida parapsilosis	86 (62.3)	86 (62.3)	1 (25.0)
Candida albicans	38 (27.5)	38 (27.5)	3 (75.0)
Other Candidas	14 (10.1)	14 (10.1)	0 (0)

Abnormalities in complete blood count

Abnormalities in complete blood count (CBC) in neonates with culture-proven sepsis overall and for different types of organisms are presented in Table 2. Just over 85% of neonates with positive blood cultures had results of CBC available and with blood for CBC having been taken at the same time or within 24 hours as a culture. Other CBCs had clotted and were repeated at more than 24 hours after the blood culture and thus were excluded from the analysis. The most common abnormality in CBC was thrombocytopenia for all pathogens, occurring in 30% of neonates with positive cultures. It was observed in lower proportion in neonates with Gram-positive compared to those with Gram-negatives (15.1% vs. 39.9%, $p < 0.001$) and Fungi (15.1% vs. 44.6%; $p < 0.001$). There were no statistical significant differences in proportion of patients with thrombocytopenia between Gram-negatives and Fungi positive cultures (39.9% vs. 44.6%; $p = 0.366$). The proportion of specific Gram-negatives with thrombocytopenia varied from 25.0% with *Pseudomonas* to 54.4% with

Klebsiella species; while there were no differences in proportions of those with thrombocytopenia amongst specific organisms that were Gram-positive or Fungi. Leukocytosis and leukopenia occurred at equal rates (15%) amongst neonates with positive cultures. Leukopenia was observed in relatively higher proportion amongst those with Gram-negatives than Gram-positive (20.8% vs 8.4%; $p < 0.001$) or fungi (20.8% vs 2.5%; $p < 0.001$); whilst leukocytosis was more common in patients with fungi compared to those with Gram-negatives (25.6% vs 13.7%; $p = 0.002$).

Abnormalities in white cell differential counts

The common abnormality in the WBC-Diff was neutrophilia for all types of organisms but it was seen in higher proportion in those with fungal infection compared to those with Gram-negatives (75.3% vs 36.7%; $p < 0.001$) and Gram-positives (75.3% vs 42.6%; $p < 0.001$) (Table 3). Neutropenia was much more common amongst the Gram-negatives than Gram-positive (20.3% vs 9.2%; $p = 0.003$) or fungi (20.3% vs 3.1%; $p < 0.001$). Overall less than 10% of neonates with culture-proven sepsis, had abnormal immature to total neutrophil ratio (ITNR). The proportions of the above abnormalities for specific organisms were similar for most organisms within each group of organisms.

CRP levels in neonates with positive blood cultures

CRP was done within 48 hours of blood culture and the results were available for 80% of patients who had positive cultures (Table 4). It was abnormal in 53.9%, 73.5%, and 82.7% of patients with positive cultures due to Gram-positives, Gram-negatives, and Candida respectively. Abnormal CRPs were of a value of greater than 40 mg/L in more than two-thirds of neonates with abnormal CRP. More than 70% of patients with Gram-negatives had abnormal CRP except for *Pseudomonas where* only 21.7% of cases had abnormal CRP. Among those with Gram positives, a higher proportion of patients with *Streptococcus agalactiae* and *Listeria monocytogenes* had high CRP of 58.8% and 86.7% respectively. More than 80% of patients with fungal culture sepsis had abnormal CRP.

Table 2 Abnormalities in complete blood count **in** neonates with positive blood cultures

	Total	**Blood count done Same time as culture n (%)**	**Leukocytosis (>25×10^9/L) n (%)**	**Leukopenia (<5×10^9//L) n (%)**	**Thrombocytopenia (<100×10^9/L) n (%)**
	886	766 (86.5)	115 (15.0)	115 (15.0)	267 (30.1)
Gram Negatives	**543**	**466 (85.8)**	**64 (13.7)**	**97 (20.8)**	**186 (39.9)**
Gram Positives	**205**	**179 (87.3)**	**20 (11.2)**	**15 (8.38)**	**27 (15.1)**
Fungi	**138**	**121 (87.7)**	**31 (25.6)**	**3 (2.48)**	**54 (44.6)**
Specific Gram Negatives					
Acinetobacter species	249	200 (80.3)	24 (12.0)	54 (27.0)	69 (34.5)
Klebsiella species	197	178 (90.4)	30 (16.9)	34 (19.1)	95 (53.4)
Pseudomonas species	30	28 (93.3)	4 (14.3)	2 (7.14)	7 (25.0)
Escherichiae coli	27	25 (92.6)	0 (0)	4 (16.0)	7 (28.0)
Enterobacter species	17	15 (88.2)	2 (13.3)	2 (13.3)	6 (40.0)
Achromobacter xylosoxidans	4	4 (100)	1 (25.0)	0 (0)	1 (25.0)
Providentia species	4	2 (50.0)	1 (50.0)	0 (0)	0 (0)
Neisseria species	3	2 (66.7)	1 (50.0)	0 (0)	0 (0)
Others	12	12 (100)	1 (8.33)	1 (8.33)	1 (8.33)
Specific Gram Positives					
Staphylococcus aureus	46	40 (87.0)	6 (15.0)	6 (15.0)	9 (22.5)
Streptococcus viridans	41	34 (82.9)	5 (14.7)	1 (2.94)	6 (17.6)
Enterococcus faecium	36	27 (75.0)	2 (7.41)	1 (3.70)	3 (11.1)
Enterococcus feacalis	31	29 (93.5)	3 (10.3)	2 (6.90)	4 (13.8)
Listeria monocytogenes	25	25 (100)	3 (12.0)	5 (20.0)	2 (8.00)
Streptococcus agalactiae	22	21 (95.5)	1 (4.76)	0 (0)	3 (14.3)
Others	4	3 (75.0)	0 (0)	0 (0)	0 (0)
Specific Fungi					
Candida parapsilosis	86	75 (87.2)	18 (24.0)	2 (2.67)	34 (45.3)
Candida albicans	38	33 (86.8)	8 (24.2	0 (0)	14 (42.4)
Other Candidas	14	13 (92.9)	5 (38.5)	1 (7.69)	6 (46.2)

Table 3 Abnormalities in white blood cell differential count among neonates with positive blood cultures

	Total	**Differential count done n (%)**	**Neutrophilia (>7.0× 10^9/L) n (%)**	**Neutropenia (<1.750×$10^{9/}$L) n (%)**	**Immature to total neutrophil ratio ≥0.2 n (%)**
	886	617 (69.6)	272 (44.1)	93 (15.1)	46 (7.5)
Gram Negatives	**543**	**379 (69.8)**	**139 (36.7)**	**77 (20.3)**	**27 (7.12)**
Gram Positives	**205**	**141 (68.8)**	**60 (42.6)**	**13 (9.22)**	**10 (7.09)**
Fungi	**138**	**97 (70.3)**	**73 (75.3)**	**3 (3.09)**	**9 (9.28)**
Specific Gram Negatives					
Acinetobacter species	249	165 (66.3)	61 (37.0)	41 (24.8)	15 (9.09)
Klebsiella species	197	145 (73.6)	56 (38.6)	24 (16.6)	10 (6.90)
Pseudomonas species	30	24 (80.0)	7 (29.2)	5 (20.8)	1 (4.17)
Escherichiae coli	27	18 (66.7)	2 (11.1)	4 (22.2)	1 (5.56)
Enterobacter species	17	10 (58.9)	5 (50.0)	2 (20.0)	0 (0)
Achromobacter xylosoxidans	4	4 (100)	2 (50.0)	0 (0)	0 (0)
Providentia species	4	1 (25.0)	1 (100)	0 (0)	0 (0)
Neisseria species	3	0 (0)	0 (0)	0 (0)	0 (0)
Others	12	12 (100)	5 (41.7)	1 (8.33)	0 (0)
Specific Gram Positives					
Staphylococcus aureus	46	31 (67.4)	13 (41.9)	5 (16.1)	1 (3.23)
Streptococcus viridans	41	28 (68.3)	12 (42.9)	3 (10.7)	2 (7.14)
Enterococcus faecium	36	18 (50.0)	7 (38.9)	0 (0)	2 (11.1)
Enterococcus feacalis	31	24 (77.4)	13 (54.2)	1 (4.17)	2 (8.33)
Listeria monocytogenes	25	21 (84.0)	9 (42.9)	3 (14.3)	3 (14.3)
Streptococcus agalactiae	22	18 (81.8)	6 (33.3)	1 (5.56)	0 (0)
Others	4	1 (25.0)	0 (0)	0 (0)	0 (0)
Specific Fungi					
Candida parapsilosis	86	62 (72.1)	42 (67.7)	3 (4.84)	4 (6.45)
Candida albicans	38	23 (60.5)	21 (91.3)	0 (0)	3 (13.0)
Other Candidas	14	12 (85.7)	10 (81.3)	0 (0)	2 (16.7)

Table 4 Abnormalities in C- reactive protein in neonates with positive blood cultures

	Total	**CRP Done, n %**	**Number with CRP ≥ 10mg/L (N =500)**			
			CRP≥ 10, n%	**CRP 10-20, n%**	**CRP 21-40, n%**	**CRP> 40, n%**
	886	708 (79.9)	500 (70.6)	77 (15.4)	88 (17.6)	335 (67.0)
Gram Negatives	**543**	**452 (83.2)**	**332 (73.5)**	**52 (15.7)**	**59 (17.8)**	**221 (66.6)**
Gram Positives	**205**	**152 (74.1)**	**82 (53.9)**	**12 (14.6)**	**18 (22.0)**	**52 (63.4)**
Fungi	**138**	**104 (75.4)**	**86 (82.6)**	**13 (15.1)**	**11 (12.8)**	**62 (72.1)**
Specific Gram Negatives						
Acinetobacter species	249	205 (82.3)	147 (71.7)	32 (21.8)	35 (23.8)	80 (54.4)
Klebsiella species	197	172 (87.3)	144 (83.7)	13 (9.03)	20 (13.9)	111 (77.1)
Pseudomonas species	30	23 (76.7)	5 (21.7)	2 (40.0)	1 (20.0)	2 (40.0)
Escherichiae coli	27	21 (77.8)	16 (76.2)	2 (12.5)	1 (6.25)	13 (81.2)
Enterobacter species	17	14 (82.4)	12 (85.7)	3 (25.0)	0 (0)	9 (75.0)
Achromobacter xylosoxidans	4	3 (75.0)	2 (66.7)	0 (0)	1 (50.0)	1 (50.0)
Providentia species	4	3 (75.0)	1 (33.3)	0 (0)	1 (100)	0 (0)
Neisseria species	3	2 (66.7)	1 (50.0)	0 (0)	0 (0)	1 (100)
Others	12	9 (75.0)	4 (44.4)	0 (0)	0 (0)	4 (100)
Specific Gram Positives						
Staphylococcus aureus	46	35 (76.1)	19 (54.3)	1 (5.26)	2 (10.5)	16 (84.2)
Streptococcus viridans	41	31 (75.6)	13 (41.9)	0 (0)	8 (61.5)	5 (38.5)
Enterococcus faecium	36	27 (75.0)	15 (55.6)	3 (20.0)	1 (6.67)	11 (73.3)
Enterococcus feacalis	31	24 (77.4)	10 (41.7)	2 (20.0)	3 (30.0)	5 (50.0)
Listeria monocytogenes	25	15 (60.0)	13 (86.7)	2 (15.4)	2 (15.4)	9 (69.2)
Streptococcus agalactiae	22	17 (77.3)	10 (58.8)	4 (40.0)	2 (20.0)	4 (40.0)
Others	4	3 (75.0)	2 (66.7)	0 (0)	0 (0)	2 (100)
Specific Fungi						
Candida parapsilosis	86	67 (77.9)	56 (83.6)	11 (19.6)	9 (16.1)	36 (64.3)
Candida albicans	38	26 (68.4)	22 (84.6)	2 (9.09)	0 (0)	20 (90.9)
Other Candidas	14	11 (78.6)	8 (72.8)	0 (0)	2 (25.0)	6 (75.0)

Abnormalities in CSF cells counts and chemistry among those with positive CSF culture

The total number of pathogens isolated from the CSF were 106 (Table 5). Cell counts were missing in 5/106 patients (4.7%) and protein levels were missing in 20/106 patients (18.9%). There were 46 (45.5 %) CSF cultures that had a CSF that was considered to be a bloody tap (defined as red blood cell count $\geq 500 \times 10^9$/L) (6). Just over a quarter of neonates (26.7%) had high CSF white cell count defined as count >20 cells/mm^3, while about two-thirds (61.6%) of neonates had protein levels >150 mg/dL. The proportion of neonates with high CSF white cell count was similar for the different types of organisms, while those with Gram-negatives (65.2%) and Candida (100%) positive cultures had high protein levels than those with Gram-positive (41.2%) ($p < 0.001$). On comparing the findings of the CSFs between those that were considered bloody taps versus those that were not bloody taps, the former group had a higher proportion with a high white cell count (32.6% vs 21.8%, $p = 0.01$) and high protein (73.5% vs 52.9%, $p < 0.001$) compared to the latter.

Table 5. White cell count and protein levels in cerebrospinal fluid (CSF) in neonates with positive CSF cultures

	All with Culture Positive Cerebrospinal Fluids (CSF) N = 106*		**Culture Positive CSF that were Bloody Taps N = 46**		**Culture Positive CSF that were not Bloody Taps N = 60**	
	Number with WCC>20/mm³	Number with Protein >150mg/dL	Number with WCC>20/mm³	Number with Protein >150mg/dL	Number with WCC>20/mm³	Number with Protein >150mg/dL
All Organisms (n = 101)	27/101 (26.7%)	53/86 (61.6%)	15/46 (32.6%)	25/34 (73.5%)	12/55 (21.8%)	27/51 (52.9%)
Gram Negatives (n = 79)	21/79 (26.6%)	43/66 (65.2%)	11/38 (28.9%)	22/28 (78.6%)	10/41 (24.4%)	20/37 (54.1%)
Gram Positives (n = 18)	5/18 (27.8%)	7/17 (41.2%)	3/5 (60.0%)	1/4 (25.0%)	2/13 (15.3%)	6/13 (46.2%)
Candidas (n = 4)	1/4 (25.0%)	3/3 (100%)	1/3 (33.3%)	2/2 (100%)	0/1 (0)	1/1/ (100%)

* - 5 cerebrospinal fluids were missing cell counts and 15 were missing protein count, WCC- white cell count

DISCUSSION

In this study, we found that all neonates who had cultures done had CBC done as part of workup for sepsis, though only 86% of CBC were available for analysis. The main reason for unavailability was that the CBC done at the time of culture had clotted. The use of CRP and WBC-Diff was relatively lower than that of CBC, at 80% and 70% respectively. An analysis of CBC the common abnormality in neonates with positive cultures was thrombocytopenia occurring in about 30% of cases and was observed at relatively higher frequencies, in infants with positive cultures due Gram-negatives and fungal isolates. The proportion of neonates with thrombocytopenia was similar between those infected with Gram-negatives and those infected with Candida. Abnormalities in total WBC occurred in only 15% of cases, with leucocytosis being more common in those with fungal sepsis and leukopenia being common in Gram-negatives. Among those with WBC-Diff available, the common abnormality was neutrophilia followed by neutropenia with neutrophilia being observed more commonly in those with fungal isolates and neutropenia being more common in those with Gram-negative isolates. Abnormalities in immature to total neutrophil ratio were observed in only less than 10% of cases. Overall the ancillary test that was abnormal in most cases with positive cultures was CRP, as it was observed to be abnormal (≥10 mg/L) in about 70% of patients who had this test done. High CSF protein was observed in a much higher proportion than high CSF cell count, occurring in about two-thirds compared to high CSF cell count that was observed in only a quarter of neonates with positive CSF cultures.

Few studies have looked at the relationship between the presence of thrombocytopenia and positive culture results. Thrombocytopenia has been observed in 49% to 67% of neonates with culture-proven sepsis (7, 8). It has been reported to be a marker of Gram-negative and fungal sepsis, with 50% and 45% of neonates with these types of sepsis having thrombocytopenia respectively (8, 9). Some studies did not observe this association (10). A multivariate analysis reported that Gram-negative sepsis, as opposed to Gram-positive sepsis, was independently associated with thrombocytopenia in neonatal sepsis (7). There are suggestions that the endotoxin produced by Gram-negative organisms causes platelet destruction, thus the high prevalence in Gram-negative infected patients. Thrombocytopenia could also indicate the severity of illness caused by Gram-negative organisms, or be due to disseminated intravascular coagulation (11-13). Based on our study, the absence of thrombocytopenia cannot be used to exclude sepsis as only about a third of neonates with

culture-proven sepsis have thrombocytopenia and its presence cannot be used to differentiate between patients infected with fungi and those with bacteria as it occurs at similar rates in those infected with Gram-negatives and those with fungal cultured confirmed sepsis.

In this study, leukocytosis and leukopenia were found to be occurring at a similar rate of 15%, with leukocytosis being observed to occur commonly among the fungal infected cases while leukopenia was observed more commonly in Gram-negatives. Other studies have shown leucocytosis to be present in 11.8% to 50% of neonates with culture-proven sepsis, thus some studies reported much higher proportion with leucocytosis than what we observed (14, 15). Leukopenia has been reported to be present in 1.8% to 6.8% of those with positive cultures (14, 16). One study reported that leukopenia was present in as high as 35% of neonates with culture-positive sepsis, reported in 40% and 30% among those with Gram-negative and Candida species respectively (17). Neutropenia has previously been reported to occur in 8.6% of infants with culture-proven sepsis, much lower than 15% reported in our study, but reported a higher prevalence of 26.6% among those with Gram-negative bacterial sepsis (18, 19). A study from Pakistan reported that I/T ratio had a sensitivity of 76.5%, much higher than the 7.5% observed in this study (15). In a retrospective review of 49 positive culture in neonates, the I/T ratio was significantly higher among Gram-negatives (19), which is a different finding from our study in which the rates of IT ratio > 0.2 occurred at similar frequencies amongst the different types of organisms. A study conducted in Japan reported similar findings to ours where the I/T ratios were similar among neonates with Gram-positive and Gram-negative bacterial growth on blood culture (20).

High CRP was the most common abnormality in neonates with positives cultures in all types of organisms. It would appear that that CRP is a relatively more reliable test in establishing the diagnosis of sepsis in neonates (21). In a cross-sectional validation study of CRP done in Pakistan in 2012, consisting of 147 neonates, with 104 having positive blood cultures, the CRP done at 72 hours of admission, had a sensitivity of 76.92% which is similar to our findings (15). The overall diagnostic accuracy of CRP in diagnosing neonatal sepsis has been reported to be 70% (22). Some have reported that the CRP was significantly high among neonates with septicaemia due to Gram-negative sepsis than in those with Gram-positive septicaemia 75.0% vs 55.3% (23). Similar to our findings, in a retrospective cross-sectional study of 303 neonates and 88 positive blood cultures, there was no significant difference with CRP positivity between the Gram-negative and Gram-positive bacteria (24). Another meta-

analysis reported CRP to have sensitivity of 62% (95% CI 52% - 72%) and concluded that the CRP level is not sufficient to accurately help in diagnosing or inform treatment decisions when a neonatal infection is suspected (25).

The common abnormality among all positive CSF cultures was high protein and the proportion with this abnormality was high amongst the Fungi and Gram-negatives. One study reported that CSF protein of > 90 mg/dL was found in 96% and of 170 mg/dL in 61% of neonates with meningitis, making this parameter a useful indicator to rule out meningitis (26). Only 27% of neonates with positive CSF culture had high CSF-WCC and the difference was not statistically significantly different among the different types of organisms. In a large retrospective study from 150 neonatal intensive care units by Pediatrix Medical Group consisting of 14017 lumbar punctures, Gram-negative meningitis was associated with high white cell counts compared to Gram-positive meningitis, a median of 1217/mm^3 vs 187/mm^3 ($p = 0.043$) (27). A traumatic tap was observed in 46% of patients with positive CSF cultures. This is similar to the prevalence of 39.5% to 46.0% reported in other studies (6, 28, 29). The proportion with high CSF WCC and CSF protein were higher in those with a bloody tap. These findings are similar to those reported by Lyons, et al which reported that the sensitivity of the CSF-WCC count was 87.9% among those with traumatic LP and 58.6% among those with non-traumatic LP (30).

The strength of the study is that there was a large number of patients with positive cultures enrolled in this study. Secondly, there was a high proportion of infants with positive cultures who had ancillary laboratory tests performed at about the same time as positive cultures and their results being available. The limitation of the study is that it is a retrospective study, thus not all ancillary test results could be analysed as some were missing. These results are from a single tertiary centre and therefore the results should be carefully considered before they are generalised to other centres as laboratory normal ranges might vary from laboratory to laboratory. Another limitation is that the demographics could not be captured, so we could not determine whether there are differences in these abnormalities between premature and term infants and also classify organisms into early and late-onset sepsis. The other limitation of this study was that there was no culture-negative group of neonates with sepsis, that could be used as a comparison group.

CONCLUSION

In conclusion, CRP test had the best sensitivity (70%) in detecting patients with culture positive neonatal sepsis. But the caveat is that a high CRP could be related to causes other than infection, and that a normal CRP would have missed about a third of those with positive cultures. The absence of abnormalities in CBC, WBC-Diff cannot be used to exclude culture-confirmed sepsis as less than 50% of patients with positive cultures have abnormalities in blood counts. Similarly, for CSF-WCC, normal CSF-WCC cannot be used to exclude positive CSF culture results as only 27% of patients with meningitis have abnormal CSF-count. Thus a decision to stop empiric antibiotics must not be based on CBC or CSF cell counts alone but also on getting culture results being negative. This highlights the importance of ensuring that cultures or other tests that can identify the organisms are performed and made available timeously and not to rely on ancillary laboratory tests only.

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APPENDICES

APPENDIX A: MANUSCRIPT SUBMITTED ACCORDING TO JOURNAL GUIDELINES

The Profile of Ancillary Laboratory Tests in Neonates with Bacterial and/or Fungal Sepsis

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Abbreviations:
CBC- Complete blood count
CHBAH - Chris Hani Baragwanath Academic Hospital
CP - Culture proven
CP-NSM - Culture proven neonatal sepsis or meningitis
CRP- C-reactive protein
CSF- Cerebrospinal fluid
HREC- Human Research Ethics Committee
I/T ratio - Immature to total neutrophil ratio
ITNR- Immature to total neutrophil ratio
NHLS - National Health Laboratory Services
NSM – Neonatal sepsis or meningitis
WBC-Diff - White blood cell differential count
WCC - White cell count

Table of Contents Summary

There is varying proportion of neonates with abnormal ancillary laboratory among those with culture-proven sepsis/ meningitis based on the type of ancillary test used/ analysed.

What's known on This Subject: Ancillary laboratory tests are used as proxy to support diagnosis of sepsis in neonates with possible serious bacterial infections, especially in culture negative sepsis. Some of the abnormalities in these tests are linked to type of infection or organism.

What This study Adds: This study shows that contribution of different ancillary laboratory tests in diagnosing neonatal sepsis varies based on type of test used as proportion of neonates with culture-proven sepsis/meningitis with abnormalities in these tests differs according to type of test assessed.

Contributors' Statement Page

Lino Sono conceptualized and designed the study, collected data, analysed data, interpreted data, drafted the initial manuscript, and revised manuscript.

Sithembiso Velaphi conceptualized and designed the study, coordinated and supervised data collection and critically reviewed the manuscript for important intellectual content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Abstract

Background
Blood and cerebrospinal fluid (CSF) cultures are used as gold standard to diagnose neonatal sepsis/meningitis. A challenge in their use is low yield, and limited availability, especially in low-resource settings. We evaluated profiles of complete blood count (CBC), c-reactive protein (CRP), and CSF cell count and protein in neonates with culture-proven sepsis/meningitis.

Methods
Neonates with positive blood and/or CSF cultures who had results of CBC, CRP, CSF cell count and protein performed within 24-48 hours of culture were enrolled. Proportion of neonates with abnormalities in these tests was determined and comparisons among different types of pathogens performed.

Results
A total of 942 isolates were cultured in blood and/or CSF. Groups of organisms isolated were Gram negatives (GN) (62.0%), Gram positives (GP) (23.4%) and Candida (14.6%). Common abnormality in CBC was thrombocytopenia, observed in 30% of neonates with culture-proven sepsis. There was a higher proportion with thrombocytopenia among GN- (39.9%) and Candida-infected (44.6%) compared GP (15.1%)($p<0.001$). Leukopenia was relatively more common among GN than GP (20.8% vs 8.4%; $p < 0.001$). Seventy percent had high CRP (≥10mg/L). Only 26.7% and 61.6% had abnormal CSF cell count (>20cells/mm^3) and high protein (>150mg/dL) among those with positive CSF cultures.

Conclusion
Majority of neonates with positive blood or CSF cultures have normal CBC or CSF cell count respectively, therefore absence of abnormalities in these parameters cannot be used solely to exclude sepsis. CRP appears to be most useful test in diagnosing sepsis as it is abnormal in 70% of patients with culture-proven sepsis.

INTRODUCTION

Sepsis has been described as a systemic inflammatory response syndrome (SIRS) to pathogens such as bacteria, viruses or fungi that is associated with haemodynamic changes and other clinical manifestations. [1-3] The definition of sepsis also includes isolation of pathogens from normally sterile body fluids such as blood and cerebrospinal fluid (CSF). Blood and/ or CSF culture is the gold standard in the diagnosis of sepsis. In resource-limited settings, healthcare facilities might not have access to blood culture results for days after working up a patient with possible serious bacterial infection (pSBI). Waiting for culture results will therefore result in delays in initiating antibiotics in neonates with pSBI. This often results in patients being treated based on clinical signs suggestive of sepsis and available laboratory ancillary tests, namely complete blood count (CBC) and/or c-reactive protein (CRP) whose results are relatively easily accessible than blood cultures. Even when blood cultures are accessible, they are only available after 24 hours and the yield is low despite a patient having clinical signs suggestive of sepsis.

Delayed initiation of appropriate treatment for sepsis is associated with increased morbidity and mortality, [4,5] thus antibiotics are started or continued based on abnormalities in CBC, white blood cell differential count (WBC-Diff) and CRP. Proportion of patients with bacterial or fungal culture-proven sepsis who have abnormal ancillary laboratory tests is not well known, especially in settings where there is high incidence of sepsis. Knowing these proportions will assist attending physicians in estimating the likelihood of patients having sepsis due to bacteria or fungal infection in settings where culture is negative or not available. In this study we sought to determine abnormalities in CBC, WBC-Diff and CRP in neonates with bacterial or fungal organisms considered pathogens isolated from blood; and to determine cerebrospinal fluid (CSF) cell count and protein abnormalities in neonates with organisms isolated in CSF.

PATIENTS AND STUDY METHODS

This was a retrospective study conducted at Chris Hani Baragwanath Academic Hospital (CHBAH), a public tertiary hospital in Soweto, South Africa. The study population were neonates with pSBI admitted to the neonatal unit at CHBAH and identified to have a positive blood and/or cerebrospinal fluid (CSF) culture from January to December 2017. The inclusion criteria consisted of all neonates who had a positive blood and/or CSF culture due to organism considered a possible pathogen and at least one of the laboratory ancillary tests results being available from the National Health Laboratory Services (NHLS) database. *Corynebacterium*, *Bacillus* and coagulase negative *Staphylococcus* were considered as contaminants and thus were excluded from the analysis. Ancillary laboratory tests assessed were total white blood cell count (WBC), platelet count, WBC-Diff, CRP levels, CSF cell count and protein. For multiple organisms cultured, each organism was considered as a separate episode of blood stream infection thus laboratory ancillary tests were captured for each separate organism. The CBCs that were assessed were only those that were done within 24 hours of culture and for CRPs it was only those that were done within 48 hours of culture. Definitions of abnormalities for different parameters on laboratory ancillary tests were as follows; leukocytosis- white blood cell count greater than 25 x 10^9/L, leukopenia- white blood cell count less than 5 x 10^9/L, thrombocytopenia- platelet count less than 100 x 10^9/L , neutrophilia- neutrophil count greater than 7 x10^9/L , neutropenia- neutrophil count less than 1.75 x10^9/L, increased immature to total neutrophil ratio- ratio equal and greater than 0.2, high CRP- CRP equal and/or greater than 10 mg/L, abnormal CSF white cell count- cell count greater than 20 cells/mm³ and high CSF protein- protein greater than 150 mg/dL. The data was captured into Excel spreadsheet and analysed using Statistica. Comparisons in proportion of patients with abnormalities in blood or CSF parameters among the different organisms were made using chi-square. The difference with p-value < 0.05 was considered

statistically significant. The study was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand.

RESULTS

Organisms isolated from sterile sites

There were 942 organisms considered pathogens that were isolated from sterile sites of neonates with pSBI over a 12 month period, 886 in blood and 106 in CSF (Table 1). Gram negatives were the most common type of organisms isolated in both blood and CSF (n = 584; 62.0 %). The most common bacteria isolated among the Gram negatives were *Acinetobacter baumannii* (n = 271; 46.4%) and *Klebsiella pneumoniae* (n = 198; 33.9%). Among the Gram positives the common bacteria were *Enterococcus* species (n = 74; 33.6%), *Staphylococcus aureus* (n = 47; 21.4%) and *Streptococcus viridans* (n = 46; 20.9%) while among the Fungi the common fungi were *Candida parapsilosis* (n = 86; 62.3%) and *Candida albicans* (n = 38; 27.5%).

Abnormalities in complete blood count

Abnormalities in complete blood count (CBC) in neonates with culture-proven sepsis overall and for different types of organisms are presented in Table 2. Just over 85% of neonates with positive blood cultures had results of CBC available and with bloods for CBC having been taken at the same time or within 24 hours as culture. Other CBCs had clotted and were repeated at more than 24 hours after the blood culture and thus were excluded from analysis. The common abnormality in CBC was thrombocytopenia for all pathogens, occurring in 30% of neonates with positive cultures. It was observed in lower proportion in neonates with Gram positives compared to those with Gram negatives (15.1% vs. 39.9%, $p < 0.001$) and Fungi (15.1% vs. 44.6%; $p < 0.001$). There were no significant differences in proportion of patients

with thrombocytopenia between Gram negatives and Fungi (39.9% vs. 44.6%; p = 0.366). The proportion of specific Gram negatives with thrombocytopenia varied from 25.0% with *Pseudomonas* to 54.4% with *Klebsiella* species; while there were no differences in proportions of those with thrombocytopenia amongst specific organisms that were Gram positives or Fungi. Leukocytosis and leukopenia occurred at equal rates (15%) amongst neonates with positive cultures. Leukopenia was observed in relatively higher proportion amongst those with Gram negatives than Gram positives (20.8% vs 8.4%; p < 0.001) or fungi (20.8% vs 2.5%; p < 0.001); whilst leukocytosis was more common in patients with fungi compared to those with Gram negatives (25.6% vs 13.7%; p = 0.002).

Abnormalities in white cell differential counts

The common abnormality in the WBC-Diff was neutrophilia for all types of organisms but it was seen in higher proportion in those with fungal infection compared to those with Gram negatives (75.3% vs 36.7%; p < 0.001) and Gram positives (75.3% vs 42.6%; p < 0.001) (Table 3). Neutropenia was much more common amongst the Gram negatives than Gram positives (20.3% vs 9.2%; p = 0.003) or fungi (20.3% vs 3.1%; p < 0.001). Overall less than 10% of neonates with culture-proven sepsis, had abnormal immature to total neutrophil ratio (ITNR). The proportions of the above abnormalities for specific organisms were similar for most organisms within each group of organisms.

CRP levels in neonates with positive blood cultures

CRP was done within 48 hours of blood culture and the results were available for 80% of patients who had positive cultures (Table 4). It was abnormal in 53.9%, 73.5% and 82.7% of patients with positive cultures due to Gram positives, Gram negatives and Candida respectively. Abnormal CRPs were of a value of greater than 40 mg/L in more than two-

thirds of neonates with abnormal CRP. More than 70% of patients with Gram negatives had abnormal CRP except for *Pseudomonas where* only 21.7% of cases had abnormal CRP. Among those with Gram positives, a higher proportion of patients with *Streptococcus agalactiae* and *Listeria monocytogenes* had high CRP of 58.8% and 86.7% respectively. More than 80% of patients with fungal culture sepsis had abnormal CRP.

Abnormalities in CSF cells counts and chemistry among those with positive CSF culture

A total number of pathogens isolated from the CSF were 106 (Table 5). Cell counts were missing in 5/106 patients (4.7%) and protein levels were missing in 20/106 patients (18.9%). There were 46 (45.5 %) CSF cultures that had a CSF that was considered to be a bloody tap (defined as red blood cell count $\geq 500 \times 10^9$/L). [6] Just over a quarter of neonates (26.7%) had high CSF white cell count defined as count >20 cells/mm³, while about two-thirds (61.6%) of neonates had protein levels >150 mg/dL. The proportion of neonates with high CSF white cell count was similar for the different types of organisms, while those with Gram negatives (65.2%) and Candida (100%) positive cultures had high protein levels than those with Gram positives (41.2%) ($p < 0.001$). On grouping the CSFs between those that were bloody taps and those that were not bloody taps, the neonates who had bloody tap CSF had a higher proportion with high white cell count (32.6% vs 21.8%, $p = 0.01$) and high protein (73.5% vs 52.9%, $p < 0.001$) than those without a bloody tap.

DISCUSSION

In this study we found that all neonates who had cultures done had CBC done as part of work up for sepsis, though only 86% of CBC were available for analysis. The main reason for unavailability was that the CBC done at the time of culture had clotted. The use of CRP and WBC-Diff was relatively lower than that of CBC, at 80% and 70% respectively. In analysis

of CBC the common abnormality in neonates with positive cultures was thrombocytopenia occurring in about 30% of cases, and was observed at relatively higher frequencies, in infants with positive cultures due Gram negatives and fungal isolates. Proportion of neonates with thrombocytopenia was similar between those infected with Gram negatives and those infected with Candida. Abnormalities in total WBC occurred in only 15% of cases, with leukocytosis being more common in those with fungal sepsis and leukopenia being common in Gram negatives. Among those with WBC-Diff available, the common abnormality was neutrophilia followed by neutropenia with neutrophilia being observed more commonly in those with fungal isolates and neutropenia being more common in those with Gram negative isolates. Abnormalities in immature to total neutrophil ratio was observed in only less than 10% of cases. Overall the ancillary test that was abnormal in most cases with positive cultures was CRP, as it was observed to be abnormal (≥10 mg/L) in about 70% of patients who had this test done. High CSF protein was observed in a much higher proportion than high CSF cell count, occurring in about two-thirds compared to high CSF cell count that was observed in only a quarter of neonates with positive CSF cultures.

There are few studies that have looked at the relationship between presence of thrombocytopenia and positive culture results. One study that used a platelet cut-off of 100 x 10^9/L to define thrombocytopenia, reported a much higher prevalence of thrombocytopenia of 67% among those with culture proven sepsis, compared to our findings where the prevalence was only 30%. [7] A prospective study of 230 positive blood cultures done in India between the year 2000 and 2005 found that the proportion of patients with fungal, Gram negatives, and Gram positive organisms who had thrombocytopenia was 50.0%, 45% and 19.6% respectively. [7] Another cohort study of VLBW with 154 positive blood cultures, reported that compared to Gram positives, those with Gram negatives and Fungi were more likely to have low platelet counts. [8] A multivariate analysis reported that Gram negative sepsis as opposed

to Gram positive sepsis was independently associated with thrombocytopenia in neonatal sepsis. [9] There are suggestions that the endotoxin produced by Gram negative organisms causes platelet destruction, thus the high prevalence in Gram-negative infected patients. Thrombocytopenia could also indicate the severity of illness caused by Gram negative organisms, or be due to disseminated intravascular coagulation. [10-12] Based on our study, absence of thrombocytopenia cannot be used to exclude sepsis as only about a third of neonates with culture-proven sepsis have thrombocytopenia and its presence cannot be used to differentiate between patients infected with fungi and those with bacteria as it occurs at similar rates in those infected with Gram negatives and those with fungal cultured confirmed sepsis.

In this study, leukocytosis and leukopenia were found to be occurring at similar rate of 15%, with leukocytosis being observed to occur commonly among the fungal infected cases while leukopenia was observed more commonly in Gram negatives. A study that used definition of leukocytosis (WBC >25 000) similar to ours, reported that 50% of positive blood culture cases had leukocytosis, [13] much higher than that we have observed. Neutropenia has previously been reported to occur in 8.6% in infants with culture proven sepsis, much lower than 15% reported in our study, but reported a higher prevalence of 26.6% among those with Gram negative bacterial sepsis. [14,15] A study from Pakistan reported that I/T ratio had sensitivity of 76.5%, much higher than the 7.5% observed in this study. [13] In a retrospective review of 49 positive culture in neonates, the I/T ratio was significantly higher among Gram negatives, [15] which is a different finding from our study in which the rates of IT ratio > 0.2 occurred at similar frequencies amongst the different types of organisms. A study conducted in Japan reported similar findings to ours where the I/T ratios were similar among neonates with Gram positive and Gram negative bacterial growth on blood culture. [16]

High CRP was the most common abnormality in neonates with positives cultures in all types of organisms. It would appear that that CRP is a relatively more reliable test in establishing the diagnosis of sepsis in neonates. [17] In a cross sectional validation study of CRP done in Pakistan in 2012, consisting of 147 neonates, with 104 having positive blood cultures, the CRP done at 72 hours of admission, had sensitivity of 76.92% which is similar to our findings.[13] The overall diagnostic accuracy of CRP in diagnosing neonatal sepsis has been reported to be 70%. [18] Some have reported that the CRP was significantly high among neonates with septicaemia due to Gram negative sepsis than in those with Gram positive septicaemia 75.0% vs 55.3%. [19] Similar to our findings, a retrospective cross-sectional study of 303 neonates and 88 positive blood cultures, there were no significant difference with CRP positivity between the Gram negative and Gram positive bacteria.[20]

The common abnormality among all positive CSF cultures was high protein and the proportion with this abnormality was high amongst the Fungi and Gram negatives. One study reported that CSF protein of > 90 mg/dL was found in 96% and of 170 mg/dL in 61% of neonates with meningitis, making this parameter a useful indicator to rule out meningitis. [21] Only 27% of neonates with positive CSF culture had high CSF-WCC and the difference was not statistically significant different among the different type of organisms. In a large retrospective study from 150 neonatal intensive care units by Pediatrix Medical Group consisting of 14017 lumbar punctures, Gram negative meningitis was associated with high white cell counts compared to Gram positive meningitis , median of 1217/mm³ vs 187/mm³ ($p = 0.043$). [22]

Traumatic tap was observed in 46% of patients with positive CSF cultures. This is similar to the prevalence of 39.5% to 46.0% reported in other studies. [6,23,24] The proportion with high CSF WCC and CSF protein were higher in those with bloody tap. These findings are similar

to those reported by Lyons, et al which reported that the sensitivity of the CSF-WCC count was 87.9% among those with traumatic LP and 58.6% among those with non-traumatic LP. [25]

The strength of the study is that there was a large number of patients with positive cultures enrolled in this study. Secondly there was a high proportion of infants with positive cultures who had ancillary laboratory tests performed at about the same time as positive cultures and their results being available. Limitations of the study is that it is a retrospective study, thus not all ancillary tests results could be analysed as some were missing. These results are from a single tertiary centre and therefore the results should be carefully considered before they are generalised to other centres as laboratory normal ranges might vary from laboratory to laboratory. Another limitation is that the demographics could not be captured, so we could not determine whether there are difference in these abnormalities between premature and term infants and also classify organisms into early and late onset sepsis.

CONCLUSION

In conclusion, CRP appears to be the most useful test in diagnosing sepsis as it is abnormal in more than two-thirds of patients with positive cultures. The absence of abnormalities in CBC, WBC-Diff cannot be used to exclude culture-confirmed sepsis as less than 50% of patients with positive cultures have abnormalities in blood counts. Similarly for CSF-WCC, normal CSF-WCC cannot be used to exclude positive CSF culture results as only 27% of patients with meningitis have abnormal CSF-count. Thus a decision to stop empiric antibiotics must not be based on CBC or CSF cell counts alone but also on getting culture results being negative. This highlights the importance of ensuring that cultures or other tests that can identify the organisms are performed and made available timeously and not to rely on ancillary laboratory tests only.

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Table 6 Pathogens isolated from blood and/or cerebrospinal fluid

	Blood or CSF N = 942	Blood N = 886	CSF N = 106
Gram Negatives	**584 (62.0)**	**543 (61.3)**	**84 (79.2)**
Acinetobacter baumannii	271 (46.4)	247 (45.5)	54 (64.3)
Klebsiella pneumonia	198 (33.9)	191 (35.2)	20 (23.8)
Pseudomonas species	32 (5.48)	30 (5.52)	2 (2.38)
Escherichiae coli	29 (4.97)	27 (4.97)	2 (2.38)
Enterobacter species	20 (3.42)	17 (3.13)	3 (3.57)
Klebsiella oxytoca	6 (1.03)	6 (1.10)	0 (0)
Achromobacter xylosoxidans	4 (0.68)	4 (0.74)	0 (0)
Providentia species	4 (0.68)	4 (0.74)	0 (0)
Neisseria species	3 (0.51)	3 (0.55)	0 (0)
Acinetobacter lwoffi	2 (0.34)	2 (0.37)	0 (0)
Others	15 (2.57)	12 (2.21)	3 (3.57)
Gram Positives	**220 (23.4)**	**205 (23.1)**	**18 (17.0)**
Staphylococcus aureus	47 (21.4)	46 (22.4)	2 (11.1)
Streptococcus viridans	46 (20.9)	41 (20.0)	5 (27.8)
Enterococcus faecium	41 (18.6)	36 (17.6)	7 (38.9)
Enterococcus feacalis	33 (15.0)	31 (15.1)	2 (11.1)
Listeria monocytogenes	26 (11.8)	25 (12.2)	1 (5.56)
Streptococcus agalactiae	23 (10.5)	22 (10.7)	1 (5.56)
Others	4 (1.82)	4 (1.95)	0 (0)
Candida and other yeasts	**138 (14.6)**	**138 (15.6)**	**4 (3.78)**
Candida parapsilosis	86 (62.3)	86 (62.3)	1 (25.0)
Candida albicans	38 (27.5)	38 (27.5)	3 (75.0)
Other Candidas	14 (10.1)	14 (10.1)	0 (0)

Table 7 Abnormalities in complete blood count in neonates with positive blood cultures

	Total	**Blood count done Same time as culture n (%)**	**Leukocytosis (>25×10^9/L) n (%)**	**Leukopenia (<5×10^9//L) n (%)**	**Thrombocytopenia (<100×10^9/L) n (%)**
	886	766 (86.5)	115 (15.0)	115 (15.0)	267 (30.1)
Gram Negatives	**543**	**466 (85.8)**	**64 (13.7)**	**97 (20.8)**	**186 (39.9)**
Gram Positives	**205**	**179 (87.3)**	**20 (11.2)**	**15 (8.38)**	**27 (15.1)**
Fungi	**138**	**121 (87.7)**	**31 (25.6)**	**3 (2.48)**	**54 (44.6)**
Specific Gram Negatives					
Acinetobacter species	249	200 (80.3)	24 (12.0)	54 (27.0)	69 (34.5)
Klebsiella species	197	178 (90.4)	30 (16.9)	34 (19.1)	95 (53.4)
Pseudomonas species	30	28 (93.3)	4 (14.3)	2 (7.14)	7 (25.0)
Escherichiae coli	27	25 (92.6)	0 (0)	4 (16.0)	7 (28.0)
Enterobacter species	17	15 (88.2)	2 (13.3)	2 (13.3)	6 (40.0)
Achromobacter xylosoxidans	4	4 (100)	1 (25.0)	0 (0)	1 (25.0)
Providentia species	4	2 (50.0)	1 (50.0)	0 (0)	0 (0)
Neisseria species	3	2 (66.7)	1 (50.0)	0 (0)	0 (0)
Others	12	12 (100)	1 (8.33)	1 (8.33)	1 (8.33)
Specific Gram Positives					
Staphylococcus aureus	46	40 (87.0)	6 (15.0)	6 (15.0)	9 (22.5)
Streptococcus viridans	41	34 (82.9)	5 (14.7)	1 (2.94)	6 (17.6)
Enterococcus faecium	36	27 (75.0)	2 (7.41)	1 (3.70)	3 (11.1)
Enterococcus feacalis	31	29 (93.5)	3 (10.3)	2 (6.90)	4 (13.8)
Listeria monocytogenes	25	25 (100)	3 (12.0)	5 (20.0)	2 (8.00)
Streptococcus agalactiae	22	21 (95.5)	1 (4.76)	0 (0)	3 (14.3)
Others	4	3 (75.0)	0 (0)	0 (0)	0 (0)
Specific Fungi					
Candida parapsilosis	86	75 (87.2)	18 (24.0)	2 (2.67)	34 (45.3)
Candida albicans	38	33 (86.8)	8 (24.2	0 (0)	14 (42.4)
Other Candidas	14	13 (92.9)	5 (38.5)	1 (7.69)	6 (46.2)

Table 8 Abnormalities in white blood cell differential count in neonates with positive blood cultures

	Total	**Differential count done n (%)**	**Neutrophilia (>7.0× 10^9/L) n (%)**	**Neutropenia (<1.750×$10^{9/}$/L) n (%)**	**Immature to total neutrophil ratio ≥0.2 n (%)**
	886	617 (69.6)	272 (44.1)	93 (15.1)	46 (7.5)
Gram Negatives	**543**	**379 (69.8)**	**139 (36.7)**	**77 (20.3)**	**27 (7.12)**
Gram Positives	**205**	**141 (68.8)**	**60 (42.6)**	**13 (9.22)**	**10 (7.09)**
Fungi	**138**	**97 (70.3)**	**73 (75.3)**	**3 (3.09)**	**9 (9.28)**
Specific Gram Negatives					
Acinetobacter species	249	165 (66.3)	61 (37.0)	41 (24.8)	15 (9.09)
Klebsiella species	197	145 (73.6)	56 (38.6)	24 (16.6)	10 (6.90)
Pseudomonas species	30	24 (80.0)	7 (29.2)	5 (20.8)	1 (4.17)
Escherichiae coli	27	18 (66.7)	2 (11.1)	4 (22.2)	1 (5.56)
Enterobacter species	17	10 (58.9)	5 (50.0)	2 (20.0)	0 (0)
Achromobacter xylosoxidans	4	4 (100)	2 (50.0)	0 (0)	0 (0)
Providentia species	4	1 (25.0)	1 (100)	0 (0)	0 (0)
Neisseria species	3	0 (0)	0 (0)	0 (0)	0 (0)
Others	12	12 (100)	5 (41.7)	1 (8.33)	0 (0)
Specific Gram Positives					
Staphylococcus aureus	46	31 (67.4)	13 (41.9)	5 (16.1)	1 (3.23)
Streptococcus viridans	41	28 (68.3)	12 (42.9)	3 (10.7)	2 (7.14)
Enterococcus faecium	36	18 (50.0)	7 (38.9)	0 (0)	2 (11.1)
Enterococcus feacalis	31	24 (77.4)	13 (54.2)	1 (4.17)	2 (8.33)
Listeria monocytogenes	25	21 (84.0)	9 (42.9)	3 (14.3)	3 (14.3)
Streptococcus agalactiae	22	18 (81.8)	6 (33.3)	1 (5.56)	0 (0)
Others	4	1 (25.0)	0 (0)	0 (0)	0 (0)
Specific Fungi					
Candida parapsilosis	86	62 (72.1)	42 (67.7)	3 (4.84)	4 (6.45)
Candida albicans	38	23 (60.5)	21 (91.3)	0 (0)	3 (13.0)
Other Candidas	14	12 (85.7)	10 (81.3)	0 (0)	2 (16.7)

Table 9 Abnormalities in C- reactive protein in neonates with positive blood cultures

	Total	**CRP Done, n %**	**Number with CRP ≥ 10mg/L (N =500)**			
			CRP≥ 10, n%	**CRP 10-20, n%**	**CRP 21-40, n%**	**CRP> 40, n%**
	886	708 (79.9)	500 (70.6)	77 (15.4)	88 (17.6)	335 (67.0)
Gram Negatives	**543**	**452 (83.2)**	**332 (73.5)**	**52 (15.7)**	**59 (17.8)**	**221 (66.6)**
Gram Positives	**205**	**152 (74.1)**	**82 (53.9)**	**12 (14.6)**	**18 (22.0)**	**52 (63.4)**
Fungi	**138**	**104 (75.4)**	**86 (82.6)**	**13 (15.1)**	**11 (12.8)**	**62 (72.1)**
Specific Gram Negatives						
Acinetobacter species	249	205 (82.3)	147 (71.7)	32 (21.8)	35 (23.8)	80 (54.4)
Klebsiella species	197	172 (87.3)	144 (83.7)	13 (9.03)	20 (13.9)	111 (77.1)
Pseudomonas species	30	23 (76.7)	5 (21.7)	2 (40.0)	1 (20.0)	2 (40.0)
Escherichiae coli	27	21 (77.8)	16 (76.2)	2 (12.5)	1 (6.25)	13 (81.2)
Enterobacter species	17	14 (82.4)	12 (85.7)	3 (25.0)	0 (0)	9 (75.0)
Achromobacter xylosoxidans	4	3 (75.0)	2 (66.7)	0 (0)	1 (50.0)	1 (50.0)
Providentia species	4	3 (75.0)	1 (33.3)	0 (0)	1 (100)	0 (0)
Neisseria species	3	2 (66.7)	1 (50.0)	0 (0)	0 (0)	1 (100)
Others	12	9 (75.0)	4 (44.4)	0 (0)	0 (0)	4 (100)
Specific Gram Positives						
Staphylococcus aureus	46	35 (76.1)	19 (54.3)	1 (5.26)	2 (10.5)	16 (84.2)
Streptococcus viridans	41	31 (75.6)	13 (41.9)	0 (0)	8 (61.5)	5 (38.5)
Enterococcus faecium	36	27 (75.0)	15 (55.6)	3 (20.0)	1 (6.67)	11 (73.3)
Enterococcus feacalis	31	24 (77.4)	10 (41.7)	2 (20.0)	3 (30.0)	5 (50.0)
Listeria monocytogenes	25	15 (60.0)	13 (86.7)	2 (15.4)	2 (15.4)	9 (69.2)
Streptococcus agalactiae	22	17 (77.3)	10 (58.8)	4 (40.0)	2 (20.0)	4 (40.0)
Others	4	3 (75.0)	2 (66.7)	0 (0)	0 (0)	2 (100)
Specific Fungi						
Candida parapsilosis	86	67 (77.9)	56 (83.6)	11 (19.6)	9 (16.1)	36 (64.3)
Candida albicans	38	26 (68.4)	22 (84.6)	2 (9.09)	0 (0)	20 (90.9)
Other Candidas	14	11 (78.6)	8 (72.8)	0 (0)	2 (25.0)	6 (75.0)

Table 10 White cell count and protein levels in cerebrospinal fluid (CSF) in neonates with positive CSF cultures

	All with Culture Positive Cerebrospinal Fluids (CSF) N = 106*		**Culture Positive CSF that were Bloody Taps N = 46**		**Culture Positive CSF that were not Bloody Taps N = 60**	
	Number with WCC>20/mm³	Number with Protein >150mg/dL	Number with WCC>20/mm³	Number with Protein >150mg/dL	Number with WCC>20/mm³	Number with Protein >150mg/dL
All Organisms (n = 101)	27/101 (26.7%)	53/86 (61.6%)	15/46 (32.6%)	25/34 (73.5%)	12/55 (21.8%)	27/51 (52.9%)
Gram Negatives (n = 79)	21/79 (26.6%)	43/66 (65.2%)	11/38 (28.9%)	22/28 (78.6%)	10/41 (24.4%)	20/37 (54.1%)
Gram Positives (n = 18)	5/18 (27.8%)	7/17 (41.2%)	3/5 (60.0%)	1/4 (25.0%)	2/13 (15.3%)	6/13 (46.2%)
Candidas (n = 4)	1/4 (25.0%)	3/3 (100%)	1/3 (33.3%)	2/2 (100%)	0/1 (0)	1/1/ (100%)

* - 5 cerebrospinal fluids were missing cell counts and 15 were missing protein count, WCC- white cell count

APPENDIX B: RESEARCH PROTOCOL

INTRODUCTION

THE PROFILE OF ANCILLARY LABORATORY TESTS IN NEONATES WITH BACTERIAL AND OR FUNGAL SEPSIS

Sepsis is a major cause of morbidity and mortality in neonates and survivors are at increased risk of neurological impairment especially in developing countries (1-3). It is classified into two categories during the neonatal period; early- and late-onset sepsis. Early-onset sepsis is defined as sepsis occurring within the first 72 hours of life and is usually due to pathogens acquired from the mother either through haematogenous route or as ascending infection through the amniotic cavity to the foetus. Late-onset neonatal sepsis is acquired from the community or healthcare system if the neonate has been admitted in a hospital. Late onset sepsis acquired from the healthcare system is defined as sepsis occurring at or after 72 hours of admission and is called healthcare associated infection.

The clinical features of sepsis include lethargy, apnoea, respiratory distress, temperature instability, feeding intolerance, abdominal distension, impaired perfusion and blood glucose instability. These features are non-specific, as they can be due to non-infectious causes. Microbial culture in blood or cerebrospinal fluid (CSF) is the gold standard for diagnosing sepsis(4). The major challenge with basing diagnosis of sepsis on culture in neonates is that culture has low sensitivity. The factors that contribute to culture having low sensitivity include inadequate volume of blood sent for culture, patients being already on antibiotics at time of blood draw and use of intra partum antibiotics (5, 6). The low sensitivity of blood culture and delays in availability of its results often necessitates that other laboratory tests are used to assist in identifying those who might be infected.

A variety of laboratory tests have been recommended to be used as ancillary tests in diagnosing neonatal sepsis. These tests include full blood count (white blood cells and differential counts, and platelets); C-reactive protein; 1,3 B-D glucan and CSF cell counts and CSF chemistry(7). The high mortality rate associated with sepsis and low yield from culture also necessitates that patients are treated with antibiotics as having sepsis based on clinical signs and abnormalities in these tests. Therefore, it would be important to determine as to how often are the ancillary tests abnormal in neonates with positive culture due to organisms considered to be pathogens. Knowing this information will assist in deciding on whether to stop or continue with antibiotics using ancillary tests as proxy for presence or absence of infection in patients who might have a false negative culture.

ANCILLARY LABORATORY TESTS USED IN DIAGNOSIS OF SEPSIS IN NEONATES

White Blood Cell Counts

The total and differential white blood cell count, absolute and immature neutrophil counts, and the ratio of immature to-total neutrophils (ITN) are used as ancillary diagnostic tests for sepsis. An ITN ratio of 0.2 or greater is suggestive of bacterial infection with a sensitivity of 60-90% (8). Abnormal ITN ratio has been reported to be more predictive of sepsis (8). Leucocytosis with a left shift is a non-specific finding that is also common in neonates with sepsis. It is recommended that white blood cell with a differential count should be performed as part of work-up for sepsis. In sepsis, total white cell count may either be low ($<5 \times 10^9$) or high ($>25 \times 10^9$), with either a low (<1750) or high (>7000) absolute neutrophil count and ITN ratio may be high (>0.2) (9). According to Manroe et al, neutrophil indices have been shown to have a high sensitivity in diagnosing early onset sepsis especially caused by group B *Streptococcus* (GBS) with a specificity of > 90% in the absence of sepsis (9, 10). However, a number of other factors have been associated with abnormal white cell counts, namely intrauterine growth restriction, metabolic disorders, drugs and asphyxia, arterial or venous sampling and maternal hypertension (9, 11).

Platelet count

Thrombocytopenia (platelet count < 150,000/μL) is the most common change found in neonates with sepsis complicating 22-35 % of neonatal ICU admissions (12). Up to 10-60 % of infants with sepsis have thrombocytopenia that can last as long as three weeks (13). During sepsis, platelets take 2-3 days to rise. Platelet count is not a reliable indicator of sepsis because there are other factors causing thrombocytopenia. Thrombocytopenia < 150,000/uL (OR 3,77 ,95% CI 1.33-10.64, $p = 0.012$) and Gram negative(as opposed to Gram positive) sepsis (OR 6.01, 95% CI 2.39-15.47 , *P 0.001*) were independently associated with mortality in neonates admitted to NICU with a septic event(14).

C - reactive protein

CRP is an acute phase reactant synthesized exclusively in the liver. CRP may be normal in the early stages of infection and values are subject to physiological variation during the first few days of life. It is secreted after 4 to 6 hours of inflammatory insult mediated by interleukin 6. The concentration doubles every 8 hours and peaks by 36 to 50 hours. Serial CRP measurements increase the test sensitivity. As a marker of bacterial infection, it has a sensitivity of 68 – 92 % and specificity of 40- 67%(15). A negative CRP at 24 to 48 has a 99% negative predictive value for diagnosis of infection(16). The CRP should be measured at

presentation and again later after 18-24 hours to facilitate decisions regarding further tests like lumbar puncture in early-onset sepsis (if initial CRP > 10mg/L) and decision making at 36 hours regarding duration of antibiotic therapy(17). However recent evidence suggest that the decision to perform and LP should be based on clinical grounds and a positive blood culture and less on inflammatory and haematological markers in isolation(18). CRP may be raised in other inflammatory conditions such meconium aspiration and asphyxia. CRP may be normal in culture positive events. A quickread CRP test value of > 16, 2 mg/L was tested as a point of care test in a resource limited hospital with a sensitivity of 84% and specificity of 88% (19).

Cerebrospinal fluid

A lumbar puncture should be done in any infant with suspected sepsis before starting antibiotics. However, the lumbar puncture can be done if the infant will not be compromised and there is a strong suspicion for sepsis and signs and symptoms of meningitis (20). The normal value for CSF varies according to gestational age, postnatal age and birth weight. In a study done in the UK by Okike et al, the pathogens were isolated from CSF alone in 26%, blood alone 22% and both in 34%. They concluded the incidence of bacterial meningitis in infants has not changed since the 1980s (21). One third of low birth weight infants with meningitis have negative blood culture. Therefore, a lumbar puncture should be performed irrespective of blood culture so as not to miss a diagnosis of meningitis.

Table 1 Cerebrospinal fluid reference for neonates (22-24).

	Preterm	Term
WBC	0-32 cells/mm^3	0-20 cells/mm^3
Proteins	> 150 mg/dL	> 150 mg/dL
Glucose	45 – 80 mg/dL	45-80mg/dL

1, 3 β-D Glucan

1, 3 βD glucan is used to diagnose and evaluate response to antifungal therapy. It is also used to diagnose fungal infection that is not in the blood stream but in other sites like the urine, peritoneum or abscesses complicating necrotizing enterocolitis (NEC). It is recommended that therapeutic decisions should be based on combining B,D glucan with clinical, radiologic and microbiological findings(25). It is supposed to decrease over time with antifungal therapy. The positive results are often difficult to interpret because of its poor specificity. The blood cultures for fungal infections are positive in approximately 50% of cases of invasive candidiasis(26). Value of 80pg/mL has sensitivity and specificity of 70.7% and 77.0% respectively with a

positive predictive values of 80.6% and a negative predictive value of 66.7%(27). The study done by Shabaan AE et al suggested a cut -off value of 99 pg/ml. The negative predictive value at this level can exclude sepsis by 90.7 %(25).

AIM

To determine the prevalence and types of abnormalities in ancillary laboratory tests used to diagnose sepsis among neonates with culture confirmed sepsis and/ or meningitis

PRIMARY OBJECTIVES

1. To determine organisms isolated from blood and /or CSF culture in neonates admitted to the neonatal unit at CHBAH.
2. To determine abnormalities in full blood count (total and differential white blood cell counts and platelet counts and I/T ratio) in neonates with pathogens isolated from blood and /or CSF culture and the proportion of neonates with these abnormalities.
3. To determine the abnormalities in C- reactive protein.
4. To determine the abnormalities in B-D Glucan
5. To determine the abnormalities in cerebrospinal fluid chemistry and /or cell counts in neonates with pathogens isolated from blood and /or CSF culture and proportion of neonates with these abnormalities
6. To compare prevalence of these tests between organisms considered to be definite pathogens to those who are considered possible pathogens or contaminants (coagulase negative *staphylococcus*)

PATIENTS AND STUDY MATERIAL

Study Design

This will be a retrospective descriptive and analytic study.

Study Setting

The study will be conducted at CHBAH, a public tertiary hospital in Soweto South Africa. The hospital admits about 5000 neonates in the neonatal unit per year. The majority of the neonates are inborn, with few who are outborn and referrals. Neonates with suspected early-onset sepsis have full blood count and blood culture done before they are started on antibiotics and CRP is done 24-48 hours after the time of initial workup in those with early-onset sepsis and at time of sepsis in those with healthcare-associated sepsis. Lumbar puncture is not routinely done during workup for early-onset sepsis unless blood culture is positive with an

organism considered to be a pathogen, but is done in those with healthcare-associated sepsis if not cardio-respiratory compromised.

Study Population

Neonates admitted in the neonatal unit at CHBAH identified to have positive blood and/or CSF culture from January to December 2017.

Inclusion criteria

Neonates who have positive blood and/ or CSF culture due to any organism considered a possible pathogen and at least one of the following test results available from the laboratory database or hospital file

a. White blood cell count
b. Platelets
c. CRP
d. CSF cell count and/ or chemistry
e. B-D glucan

If the same organism is cultured within 7 days, it will be considered as single episode. For multiple organisms cultured, each will be considered as a separate episode of blood stream infection and patient information will be entered for each separate organism.

Exclusion criteria

Organism culture considered a definite contaminant namely; *Corynebacteria* and *Bacillus* for both early and late-onset sepsis and Coagulase negative *staphylococcus* for early-onset sepsis

Study procedures

A list of names of neonates admitted in the neonatal unit at CHBAH with positive blood culture results will be obtained from NHLS laboratory computerized database. These names with their hospital numbers will be used to get these patients' results of total and differential white cell and platelet counts, CRP, BD glucan, CSF cell count and CSF chemistry done on the day or within 3 days after the positive blood culture results, will be retrieved. Abnormalities in the abovementioned tests will be assessed for each patient. Proportion of neonates with abnormalities in these tests will be calculated for all patients with positive cultures and for patients stratified according to the type of organism (Gram positive bacteria, Gram negative and Candida) and name of organism. Comparisons of proportions of neonates with abnormalities in the groups of tests will be made between different types and names of organisms. Patient's hospital files will be retrieved and reviewed for demographic characteristics.

Data collection

Data to be collected will include names and types of organism cultured, site from which the organism was identified, total white blood cell count, absolute neutrophil count, platelet count, CRP, B-D glucan, CSF polymorphs count, CSF lymphocyte count, CSF protein and CSF glucose. From the patient files the following information will be extracted: birth weight, gestational age, sex, mode of delivery, HIV exposure, Apgar score, age at isolation of organism and outcome at discharge.

Definitions of abnormal tests

Leukocytosis -Total white blood cell count of more than 30 $x10^9$/L in neonates <72 hours and more than 25 $x10^9$/L in neonates ≥ 72 hours old(9).

Leukopenia: Total white blood cell count of less than 9 $x10^9$/L in neonates <72 hours and less than 5 $x10^9$/L in neonates ≥ 72 hours old(9).

Neutropenia – Absolute neutrophil count of < 1750 ul^{-1}(9).

Neutrophilia – Absolute neutrophil count of >7000 ul^{-1}(9, 28).

Thrombocytopenia – Platelet count < 150 000u/L(12, 14).

Immature to total neutrophil count ratio- > 0.20(9, 29).

High CRP- a CRP value of >10mg/L(17).

High B-D glucan- a value of > 80pg/ml(27).

Data Analysis

Data will be captured into Excel spread sheet and analysed using Statistica. All laboratory tests will be presented as either normal or abnormal using cut-off points as presented under in *Table 1 or Definition*s. Comparisons in abnormalities types of organisms will be analysed using Student t-tests and chi-square for continuous and dichotomous variables respectively. Differences with p-value <0.05 will be considered statistical significant.

ETHICS

Risks: The trial is retrospective; there is no risk for participants

Confidentiality: Identifiers will be used to get the results and to retrieve files. After getting the results and files, identifiers will be kept separately from the rest of the data. The participants in the study will be identified for study purposes with a unique numerical identifier that will be kept confidential. The information will be kept on a password protected computer.

Cost to participants: Participants will not incur any cost. The information will be retrieved from the medical records and laboratory.

Ethics committee approval: The CHBAH medical advisory Committee will be approached for permission to conduct the study. The protocol will be approved by the Human Research Ethics committee (HREC) at the University of the Witwatersrand.

Permissions: Permissions will be sought from NHLS to access the microbiology database to identify patients who had blood or CSF positive cultures from the neonatal wards at CHBAH and to retrieve FBC, CRP, CSF and B-D glucan results. Permission will also be sought from Head of Department of Paediatrics at CHBAH to access patients' files.

TIME FRAMES FOR STUDY CONDUCT

	Nov 17-Jan 2018	Feb 2018	Mar- -Apr 2018	May- Jul 2018	July-Aug 2018	Sept-Oct 2018	Nov 2018
Developing Protocol	[redacted]						
Submission to Ethics and Postgraduate Committee		XXX					
Responding to comments by protocol review Committee			XXX				
Data Collection				XXX			
Data Analysis					XXX		
Writing Manuscript						XXX	
Submission for publication/ Marking							XXX

FUNDING

Costs for the study will be incurred by the investigator and these will be for stationery.

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APPENDIX C: DATA CAPTURING SHEET

Study Number: _____

Demographic Details:

Maternal age: _____; Gravida: _____; Maternal HIV: _____

Maternal Illness: _____

Mode of Delivery: Vaginal/ Abdominal/NR; MSL: Yes/No/NR

Gestational age (weeks): _____; Birth weight (grams): _____; Sex: F/M/ NR;

Date of birth: _____; Apgar score at 5 min: _____; Place of birth: _____

Laboratory Results

Date Blood culture Taken: _____; ***Organism isolated***: _____

FBC:

Date FBC taken: _____; Within how many days from culture date: _____

Total Wcc: ____;Total Abs. Neutr.:_____; Total Abs. Imm. Neutr.:_____;
ITNR:____;Platelets:_____

Date CRP taken: _____; CRP Results: _____;

B-D Glucan done: **Yes/ No**; if yes Date B-D Glucan taken: _____; B-D Glucan Results:_____

Cerebrospinal Fluid:

Lumbar puncture done: **Yes/ No**, if yes complete details below

Date CSF Taken: _____

Polys: _____; Lymphs: _____; rbcs: _____

Protein: _____; Glucose: _____; Serum glucose: _____

CSF culture positive: **Yes/ No**; if Yes, state name of Organism:

APPENDIX D: ETHICS CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Lino Sono

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180289

NAME: (Principal Investigator)	Dr Lino Sono
DEPARTMENT:	Paediatrics - Neonatology Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	The profile of ancillary laboratory tests in neonates with bacterial and or fungal sepsis
DATE CONSIDERED:	23/02/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Sithembiso Velaphi
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	21/06/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **February** and will therefore be due in the month of **February** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX E: TURNITIN REPORT

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Pediatrics considers unsolicited manuscripts in the following categories: reports of original research, particularly clinical research; review articles; special articles; and case reports. When preparing a manuscript for *Pediatrics*, authors must first determine the manuscript type and then prepare the manuscript according to the specific instructions below.
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Relevance to readers is of primary importance in manuscript selection. The readership includes general and specialist pediatricians, pediatric researchers and educators, and child health policy-makers. *Pediatrics* receives many more high quality manuscripts than can be accommodated based on our available space. The current acceptance rate is approximately 10%. An article that is thought by the editors to be not relevant to readers, outside of scope or very unlikely to be accepted may be rejected without review. All manuscripts considered for publication are peer reviewed. Peer reviewers are selected by the editors based on their expertise in the topic of the manuscript; generally at least 2 reviews are required before a decision is rendered. Authors may suggest appropriate reviewers and may also suggest reviewers who should not review the manuscript.
Authors should carefully follow instructions for manuscript preparation, and ensure that the manuscript is proofread before submission. Manuscripts that do not adhere to the author instructions will not be considered for review. Careless preparation of a manuscript suggests careless execution of the research and therefore makes acceptance unlikely. Manuscripts are scanned for plagiarism using the latest software; if potential plagiarism is detected, the editors will contact the authors for clarification, and may also contact the authors' institution.

Submissions of original research are judged on the importance and originality of the research, scientific strength, clinical relevance, the clarity of the manuscript, and the number of submissions on the same topic. *Pediatrics* does not publish manuscripts that involve animal research.
Pediatrics accepts review articles, with preference given to systematic reviews, which may include meta-analyses. State-of-the-Art Review Articles and Perspectives are generally solicited by the editors or the associate editors for their respective sections. Special Articles reflect topics or issues of relevance to pediatric health care that do not conform to a traditional study format. Case Reports must challenge an existing clinical or pathophysiologic paradigm; provide a starting point for novel hypothesis-testing clinical research; and/or focus on topics pertinent to the pediatric generalist. Quality Reports provide a venue for manuscripts that describe the implementation and outcome of quality-improvement projects. Authors should review and follow the comprehensive reporting guidelines for a wide variety of study designs that are available at http://www.equator-network.org/home/.
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Publication Ethics

Authorship. An "author" is someone who has made substantive intellectual contributions to a published study. Each author is required to meet ALL FOUR of the following criteria:

1. Substantial contribution(s) to conception and design, acquisition of data, or analysis and interpretation of data; **and**
2. Drafting the article or revising it critically for important intellectual content; **and**
3. Final approval of the version to be published, **and**
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Journal Style

All aspects of the manuscript, including the formatting of tables, illustrations, and references and grammar, punctuation, usage, and scientific writing style, should be prepared according to the most current *AMA Manual of Style* (http://www.amamanualofstyle.com).[1]

Author Listing. All authors' names should be listed in their entirety, and should include institutional/professional affiliations and degrees held.

Authoring Groups. If you choose to include an organization, committee, team, or any other group as part of your author list, you must include the names of the individuals as part of the Acknowledgments section of your manuscript. This section should appear after the main text prior to your References section. (If your Acknowledgments includes both group members *and* other persons/organizations who are not in that group, you should instead list the group members in a separate appendix to avoid confusion.) The terms "for" or "on behalf of" must also be used when referencing the authoring group in the by-line.

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Clinical Trials

A study is considered a clinical trial if it prospectively assigns human subjects (whether randomized or not) to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like.

If authors report the results of a clinical trial, they must affirm that the study has been registered at www.clinicaltrials.gov or another WHO-approved national or international registry prior to the enrollment of the first subject. Information on requirements and appropriate registries is available at www.icmje.org. The trial registration number must be listed on the title page, and at the end of the abstract.
All articles reporting results of clinical trials must include the Data Sharing Statement on their Title Page.
Authors are also required to complete both pages of a CONSORT Form (flowchart and checklist) and submit these with their manuscript. In our submission system, these files appear under "Instructions and Forms". For observational epidemiological studies, follow the appropriate STROBE checklist.

- Download a CONSORT form checklist (PDF) here.
- Download a CONSORT form flowchart (PDF) here.

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Reuse of Data Sets

If a manuscript uses the same or similar data contained in previously published articles, the authors must state this in the cover letter (and provide citations to the related or possibly duplicative materials).

If a separate manuscript by the same authors using the same data set is under review or accepted but not yet published in another journal, the authors must state this in the cover letter and provide enough information to assure that the manuscript submitted to *Pediatrics* is not duplicative.
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Data Sharing

The International Committee of Medical Journal Editors (ICMJE) requires ICMJE journals to include data sharing statements in articles that report results of clinical trials.

Data sharing statements must include:

- Whether deidentified participant data (including data dictionaries) will be shared
- The data that will be shared
- Whether additional documents will be made available
- The start and end dates of data availability
- Access criteria
- How the data will be made available

The data sharing statement must be included on the title page of your manuscript and entered into the section provided in the manuscript management system.

If you will not be sharing your data, insert the following statement on your title page and in the manuscript submission system.
Data Sharing Statement: Deidentified individual participant data will not be made available.
If you will be sharing your data, refer to the table in the data sharing section of the ICMJE clinical trials page for examples of how to incorporate the required information into your statement, and refer to the example below.

Data Sharing Statement: Deidentified individual participant data (including data dictionaries) will be made available, in addition to study protocols, the statistical analysis plan, and the informed consent form. The data will be made available upon publication to researchers who provide a methodologically sound proposal for use in achieving the goals of the approved proposal. Proposals should be submitted to _____[INSERT EMAIL ADDRESS OR OTHER CONTACT INFORMATION].

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Formatting Requirements

All submissions must adhere to the following format:

- Times New Roman font, size 12, black
- Title Page, Contributors' Statement Page, Abstract, Acknowledgments, and References should be **single-spaced**
- Only the Main Body Text should be **double-spaced**
- Main Submission Document as Microsoft Word or RTF file (no PDFs)
- Do **not** include page headers, footers, or line numbers in new submissions.
- Do **not** include footnotes within the manuscript body. Footnotes are allowed only in tables/figures.

Refer to the "Article Types" section for specific guidelines on preparing a manuscript in each category. Note in particular the requirements regarding abstracts for different categories of article.

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Title Page

The "title page" should appear first in your manuscript document, and depending on the individual needs of a paper may encompass more than one page.

Title pages for all submissions **must** include the following items (as shown in the sample Title Page):

1. **Title** (97 characters [including spaces] or fewer)
2. **Author listing.** Full names for all authors, including degrees, and institutional/professional affiliations. These affiliations should list the institution where the research presented in the article took place; if the affiliation has changed, add a note indicating the additional affiliation. *Pediatrics* permits a statement of equal contribution for two first authors only; on the title page, includes asterisks by each name and a statement that reads: * *Contributed equally as co-first authors.*
3. **Corresponding Author.** Contact information for the Corresponding Author (including: name, address, telephone, and e-mail). Again, note that the affiliation should list the institution where the research presented in the article took place; if the affiliation has changed, add a note indicating the additional affiliation. *Pediatrics* allows one Corresponding Author only; the position of Corresponding Author does not imply seniority or any other status.
4. **Short title** (55 characters [including spaces] or fewer). Please note: the short title may be used on the cover of the print edition.
5. **Financial Disclosure Statement** for all authors. Disclose any financial relationships that could be broadly relevant to the work. If none, say "Financial Disclosure: The authors have no financial relationships relevant to this article to disclose."
6. **Funding source**. Research or project support, including internal funding, should be listed here; if the project was done with no specific support, please note that here. Technical and other assistance should be identified in Acknowledgments. If your funding body has open access requirements, please contact the Editorial Office prior to submission. Pediatrics has a 12 month embargo on articles (followed by a 4 year open access period) and does not allow articles to be opened for Creative Commons or similar licenses.

7. **Conflict of Interest Statement** for all authors. If none, say “Potential Conflicts of Interest: The authors have no conflicts of interest relevant to this article to disclose.”
8. **If applicable, Clinical Trial registry name, registration number, and data sharing statement**. We adhere to ICMJE guidelines, which require that all trials must be registered with ClinicalTrials.gov or any other WHO Primary registry. All articles reporting results of clinical trials must also include the Data Sharing Statement.
9. **Abbreviations**. List and define abbreviations used in the text. If none, say "Abbreviations: none".
10. **Table of Contents Summary.** All articles with abstracts require this summary. This brief summary is limited to 25 words. For accepted manuscripts, this will appear under the author names in the table of contents to give the reader a brief insight into what the article is about. It should entice the reader to read the full article. For example: *"Through linkage of state Medicaid and Child Protective Services databases, this study captures similarities and differences in health care expenditures based on a history of child maltreatment."*
11. For Regular Article submissions, include both the **“What’s Known on This Subject"** and the **"What This Study Adds”** summaries (see below under Regular Article type for description). These are not needed for any other article type.

If a title page does not include all of the above items, the submission may be returned to the authors for completion.

- **Download and view a sample Title Page (PDF)** here.

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Contributors' Statement Page

All submissions (excluding Commentaries) must contain a Contributors’ Statement Page, directly following the Title Page(s) and in the specific format described below. Manuscripts lacking a properly formatted Contributors' Statement Page will be returned to the authors for correction.

All persons designated as authors must qualify for authorship (see "Publication Ethics" above), and all those who qualify should be listed. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. The Contributors' Statement Page lists the authors and specifies the contribution(s) made by each individual. If multiple individuals have identical contributions they may be listed together; do not list an author more than once.

You must follow the required format when creating your Contributors’ Statement Page or your manuscript will be returned for correction.

- Each author should only appear once.
- Use names, not initials.
- If multiple authors have identical contributions, you can list them in the same sentence; otherwise, list each author separately.
- Conclude your statement by confirming that: All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Sample Contributors' Statement:

Dr Smith and Prof Jones conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript.

Drs Brown, Grey, and Black and Ms Johnson designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.

Dr Green conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Note: Contributors who do not meet the criteria for authorship (such as persons who helped recruit patients for the study, or professional editors) should be listed in an Acknowledgments section placed after the manuscript’s conclusion and before the References section. Because readers may infer their endorsement of the data and conclusions, these persons must give written permission to be acknowledged. These permissions do not need to be submitted with the manuscript unless requested by the editors.

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Word Count

To determine article length, count the body of the manuscript (from the start of the Introduction to the end of the Conclusion). The title page, contributors' statement page, abstract, acknowledgments, references, figures, tables, and multimedia are not included.

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Figures, Tables, and Supplementary Material

For any figure, table, or supplementary material reproduced or adapted from another source, authors are required to obtain permission from the copyright holder, and proof of permission must be uploaded at the time of submission. The legend must include a statement that the material was used or adapted with permission.

Figures

Authors should number figures in the order in which they appear in the text. Figures include graphs, charts, photographs, and illustrations. Each figure must include a legend (placed as a list appearing after the References) that does not exceed 50 words. Abbreviations previously expanded in the text are acceptable. Upload figures as separate files; list figure legends as the last item in your main Word/text file. *Do **not** paste figures into your manuscript text/Word file.* There is no maximum number for figures.
Figure arrays should be clearly labeled, preassembled, and submitted to scale. Figure parts of an array (A, B, C, etc.) should be clearly marked in capital letters in the upper left-hand corner of each figure part.

Technical requirements for figures: The following file types are acceptable: TIFF, PDF, EPS, and PNG. Color files must be in CMYK (cyan, magenta, yellow, black) mode.
Pediatrics ***cannot*** accept Excel or PowerPoint files for any part of your submission. To repeat: upload figures as separate files; list figure legends as the last item in your main Word/text file. *Do **not** paste figures into your manuscript text/Word file.*
Style for figures: Readers should be able to understand figures without referring to the text. Avoid pie charts, 3-dimensional graphs, and excess ink in general. Make sure that the axes on graphs are labeled, including units of measurement, and that the font is large enough to read. Generally delete legends or other material from the graph if it makes the picture smaller. Color graphs should be interpretable if photocopied in black and white.

Tables

Tables should be numbered in the order in which they are cited in the text and include appropriate headers. Tables should not reiterate information presented in the Results section, but rather should provide clear and concise data that further illustrate the main point. Tabular data should directly relate to the hypothesis. Table formatting should follow the current edition of the *AMA Manual of Style*. There is no maximum number of tables.
Technical requirements for tables: Tables should be constructed using a Microsoft Word program and inserted in numerical order at the end of the manuscript, either within the main Word document (following the references) or as separate files. Do not provide tables in scan/image format. *Pediatrics cannot accept Excel or PowerPoint files for any part of your submission.* There is no maximum number for tables.
Style for tables: Tables should be self-explanatory. Avoid abbreviations; define any abbreviations in footnotes to the table. Avoid excess digits and excess ink in general. Where possible, rows should be in a meaningful order (e.g., descending order of frequency). Provide units of measurement for all numbers. In general, only one type of data should be in each column of the table.
Presentation of Numbers and Statistics

- Results in the abstract and the paper generally should include estimates of effect size and 95% confidence intervals, not just P- values or statements that a difference was statistically significant.
- Statistical methods for obtaining all P-values should be provided
- Units of independent variables must be provided in tables and results sections if regression coefficients are provided
- Authors should avoid expressing effect sizes in the form of highly derived statistics.

Equations should be typed exactly as they are to appear in the final manuscript. The following table, adapted from the guidelines for authors for the *Annals of Internal Medicine* by editors of *Medical Decision Making*, shows how to present certain percentages and some statistical measures:

Supplemental Information

Authors may wish to include additional information as part of their article for inclusion in the online edition of *Pediatrics*. References to any online supplemental information must appear in the main article. Such supplemental information can include but are not limited to additional tables, figures, videos, audio files, slide shows, data sets (including qualitative data), and online appendices. If your study is based on a survey, consider submitting your survey instrument or the key questions as a data supplement. Authors are responsible for clearly labeling supplemental information and are accountable for its accuracy. Supplemental information will be peer reviewed, but not professionally copyedited.

Videos

Pediatrics encourages the submission of videos to accompany articles where relevant. Links can be placed in the article for use when it is accessed electronically. All videos must adhere to the same general permission rules that apply to figures (i.e.: parental consent when a patient is identifiable).

All videos should be submitted at the desired reproduction size and length. To avoid excessive delays in downloading the files, videos should be no more than 6MB in size, and run between 30 and 60 seconds in length. In addition, cropping frames and image sizes can significantly reduce file sizes. Files submitted can be looped to play more than once, provided file size does not become excessive. Video format must be either .mov or .mp4.

Authors will be notified if problems exist with videos as submitted, and will be asked to modify them if needed. No editing will be done to the videos at the editorial office—all changes are the responsibility of the author.

Video files should be named clearly to correspond with the figure they represent (i.e., figure1.mov, figure2.mp4, etc.). Be sure all video files have filenames that are no more than 8 characters long and include the suffix “.mov” or ".mp4." A caption for each video should be provided (preferably in a similarly named Word file submitted with the videos), stating clearly the content of the video presentation and its relevance to the materials submitted.

IMPORTANT: One to four traditional still images from the video **must** be provided. These still images may be published with the article and will act as thumbnail images in the electronic edition that will link to the full video file. Please indicate clearly in your text whether a figure has a video associated with it, and be sure to indicate the name of the corresponding video file. A brief figure legend should also be provided.

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Regular Article

Abstract length: 250 words or fewer (structured, as noted below)
Article length: 3,000 words or fewer
Regular Articles are original research contributions that aim to inform clinical practice or the understanding of a disease process. Regular Articles include but are not limited to clinical trials, interventional studies, cohort studies, case-control studies, epidemiologic assessments, and surveys. Components of a Regular Article include:

- **What's Known on This Subject**
- **What This Study Adds**

These two brief summaries are each limited to 40 words. Please use precise and accurate language in paragraph form (i.e., not bullet points). For manuscripts accepted as Regular Articles, these summaries will become a highly visible part of your published paper, with prominence on the first page. Moreover, these summaries may be highlighted and presented in other areas of the journal. It is therefore paramount that you use language of the same caliber as the rest of your paper.

- **Structured Abstract (four paragraphs with headings in boldface type; single-spaced)**

The abstract should consist of: Background (or Objectives, or Background and Objectives), Methods, Results, and Conclusions. The Objective should clearly state the hypothesis; Methods, inclusion criteria and study design; Results, the outcome of the study; and Conclusions, the outcome in relation to the hypothesis and possible directions of future study.

- **Body of Article**

For the body of your article, follow this general outline:

- Introduction

A 1- to 2-paragraph introduction outlining the wider context that generated the study and the hypothesis.

- Patients and Methods

This section should detail inclusion criteria and study design to ensure reproducibility of the research. All studies that involve human subjects must be approved or deemed exempt by an official institutional review board; this should be noted here.

- Results

This section should give specific answers to the aims or questions stated in the introduction. The order of presentation of results should parallel the order of the methods section.

- Discussion

The section should highlight antecedent literature on the topic and how the current study changes the understanding of a disease process or clinical situation, and should include a section on the limitations of the present study.

- Conclusion

A brief concluding paragraph presenting the implications of the study results and possible new research directions on the subject.

General submission instructions (including cover letter, title page requirements, contributors' statement page, journal style guidance, and conflict of interest statements) apply to Regular Articles.

- **Download and view a sample Regular Article manuscript (PDF)** here**.**

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Advocacy Case Studies

Abstract length: 250 words or fewer (unstructured: no heading, single paragraph)
Article length: 3,000 words or fewer

Author limit: Four (4) (All authors need to have been engaged in the advocacy work being described in the case study. Additional authors can be added with permission of the editors.)
Advocacy Case Studies describe a specific, organized effort in child advocacy that results in changes to systems that affect child health and wellbeing.

These reports should focus on the advocacy process and outcomes of the intervention, not the evidence that underlies the advocacy work. We encourage reports that provide lessons that others could adopt.

Authors should follow the following format.

Introduction
What was the problem? Describe the local environment, situation, and motivation for the advocacy work. What was the overall goal of the advocacy work? Be specific and include objectives.
Methods and Process
Who was involved in the advocacy work? Describe the stakeholders involved and how they were brought together. What was the approach of the advocacy work? Describe the challenges faced and how they were addressed. How was success defined and measured? What sources of assistance or support was central to the advocacy work?
Outcomes
What were the results of your advocacy? Link these back to the goals and objectives. Describe any communication of these results if integral to sustaining the project.
Lessons Learned
What are the lessons learned from the advocacy work that are relevant for pediatricians and other child health care providers?
Conclusions
How will your advocacy work be sustained? Describe any future plans.
The general submission instructions (including cover letter, title page, contributors' statement page, journal style guidance, and conflict of interest statements) also apply to Case Reports.

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Case Report

Abstract length: 250 words or fewer (unstructured: no headings, run in a single paragraph)
Article length: 1,600 words or fewer
Author limit: Seven (7) authors or fewer
Case Reports highlight unique presentations or aspects of disease processes that may expand the differential diagnosis and improve patient care. In general, case reports will include 10 cases or fewer. For a manuscript to be considered a Case Report, it must meet at least one of the following three criteria:

1. Challenge an existing clinical or pathophysiologic paradigm.
2. Provide a starting point for novel hypothesis-testing clinical research.
3. Focus on topics pertinent to the pediatric generalist, allowing pediatrics colleagues to provide improved care. (Manuscripts meeting this criterion will be prioritized over other submissions.)

Case Reports should consist of an unstructured abstract that summarizes the case(s), a brief introduction (recommended length, 1-2 paragraphs), a section that details patient presentation, initial diagnosis and outcome, as well as a discussion that includes a brief review of the relevant literature and describes how this case brings new understanding to the disease process.

Authors may find the criteria for case reports as contained in the CARE guidelines useful in preparing their manuscript.
Written consent must be obtained from the parent or guardian. You do not need to include a copy with your submission unless the patient may be identifiable; however, a copy must be provided to *Pediatrics* upon request. *Pediatrics* does not supply a consent form.

The general submission instructions (including cover letter, title page, contributors' statement page, journal style guidance, and conflict of interest statements) also apply to Case Reports. Do **not** include "a case report" or similiar language in your title as this is redundant; published manuscripts will appear in the Case Reports section.

- **Download and view a sample Case Report manuscript (PDF)** here**.**

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Commentary

Abstract length: no abstract
Article length: 400 to 800 words
Commentaries are opinion pieces consisting of a main point and supporting discussion. These contributions usually pertain to and are published concurrently with a specific article; the commentary serves to launch a broader discussion of a topic. Commentaries may address general issues or controversies in the field of pediatrics.

Commentaries are solicited by the editors. Unsolicited opinion pieces are published as Pediatrics Perspectives. **Responses to published articles should be submitted as online Comments.**
The general submission instructions (including cover letter, title page, contributors' statement page, journal style guidance, and conflict of interest statements, also apply to commentaries); commentaries do not require a Contributors' Statement Page.

- **Download and view a sample Commentary manuscript (PDF)** here**.**

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Diagnostic Dilemmas and Clinical Reasoning

Abstract length: 250 words or fewer (unstructured: no headings, run in a single paragraph)
NOTE: ***Abstracts must not reveal the final diagnosis***
Article length: 3,500 words or fewer
Author limit: Seven (7) authors or fewer
Diagnostic Dilemmas and Clinical Reasoning articles are interactive case studies, with comments inserted by generalists and specialists asked to comment on the case, simulating what might occur in an oral case presentation.

The goal of this feature is to present clinical cases that are diagnostic dilemmas and that involve the input of both generalists and subspecialists who comment as segments of the case are presented, similar to Ethics Rounds feature articles. Each case presented should generate a dialogue about unusual or complicated disease processes and stimulate discussion about clinical reasoning. The initial case description should include the chief complaint and enough information to generate an initial differential diagnosis. Clinical details should alternate with input from generalists and from subspecialists as the case evolves and as the ultimate diagnosis is made. The case should culminate with a brief (750–1,000) summary of the key points of the case and of the ultimate diagnosis. Use of media, such as radiology studies, pathology specimens, or video clips as, is encouraged to complement the discussion.

- Authors may come from any institution. The case may be one that was discussed in the hospital's teaching rounds (many hospitals have sessions entitled Case Conference, CPC, Professorial Rounds, or something similar).
- Manuscripts will be submitted for peer review, with acceptance contingent on positive peer reviews and input from the editorial board.
- All cases should be real cases. On submission, authors must attest that they have written consent from the family. Written consent from the family and from the providers who cared for the patient is also required before a manuscript can be published (the consent can be in the form of an email); when uploading consent, select the 'Supplemental File NOT for Review' type. Instances where there are extenuating circumstances in which family consent may be problematic will be handled on a case-by-case basis. If a case is

published without family consent, enough elements should be changed so that the patient and family are not recognizable. If the case is too unique to be disguised, then those involved in the care of the patient cannot be authors, and the published paper must have no link to the institution where the case took place.
- The requirements of local institutional review boards should be followed.
- Authorship: All authors must fulfill the ICJME criteria for authorship.

Questions can be addressed to Andrea Cruz, MD, MPH, section editor for Diagnostic Dilemmas and Clinical Reasoning, here.

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Ethics Rounds

Ethics Rounds present discussions of cases that illustrate ethical dilemmas in patient care, research, or administration. Authors who have a case that raises ethical issues and who want to submit a paper for Ethics Rounds should email Assistant Editor John Lantos (email).
The general submission instructions (including cover letter, title page, contributors' statement page, journal style guidance, and conflict of interest statements, also apply to Ethics Rounds).

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Family Partnerships

Abstract length: No abstract
Article length: 2,000 words or fewer
Author limit: Four (4) authors or fewer
Reference limit: 10 references or fewer
Pediatrics is interested in publishing articles that reflect the joint perspective of patients, families, and the health care professionals taking care of the family and child. These articles should be written collaboratively and reflect their shared thoughts about a topic related to children's health care. Examples of topics that articles could address include shared decision-making, use of the Internet or other technologies to improve care, family-centered rounds, health care disparities, or issues related to medical education. These are just examples; the Executive Editorial Board would be willing to consider any relevant manuscript as long as it represents the voices of patients/families and healthcare providers. The manuscript should reflect a partnership amongst the authors.
If an individual patient's story is to be shared as a narrative, the article should not just focus on that patient's story and what went right or wrong, but reflect a broad perspective so that the lessons learned can be generalizable to others. The audience for these articles will primarily be health care professionals, but these articles will also be made free to the public so everyone can potentially benefit from reading the manuscript.

Specific points to consider: It would be acceptable for authors to write sections individually from their unique viewpoint. The article should contain a jointly written introduction and conclusion to ensure an overall collaborative voice.

Specific questions may be directed to Lewis First, MD, editor in chief of *Pediatrics* at lewis.first@uvm.edu.

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Monthly Feature

Abstract length: no abstract
Article length: 1,200 words or fewer
The Monthly Feature column offers an opportunity to gain insight into aspects of our field: past, present, and future. Alternating monthly, the column will provide ongoing updates from four standing groups: (1) Global Health; (2) the Council on Medical Student Education in Pediatrics

(COMSEP); (3) the Section on Pediatric Trainees (SOPT); and (4) the Historical Archives Advisory Committee for the AAP.

While many of the Monthly Features are invited, any queries or proposals should be directed to the editors of their respective columns: Jay Berkelhamer, MD (contact) for Global Health; Robert Dudas, MD (contact) for COMSEP; Hannah Rosenblum, MD (contact) for SOPT; and Jeffrey Baker, MD (contact) for the AAP Historical Archives Advisory Committee.
The general submission instructions (including cover letter, title page, contributors' statement page, journal style guidance, and conflict of interest statements, also apply to Monthly Features); Monthly Features do not require an abstract.

- **Download and view a sample Monthly Feature manuscript (PDF)** here**.**

SOPT Monthly Feature
This section publishes insightful updates and opinion articles on all aspects of pediatrics, written from the unique perspective of the trainee.

The goal of the editorial board of the AAP Section on Pediatric Trainees (SOPT) Monthly Feature is to work with trainee authors to develop thoughtful and timely articles related to pediatrics that appeal to everyone from medical students to well-seasoned practitioners. Topic content that focuses on training in pediatric medicine is preferred, but a range of other content areas will be considered. Topics should be relevant to students, residents and fellows, but also of general interest to the readership of *Pediatrics*. The issue being discussed must be *uniquely viewed from the trainee's perspective*, not from that of the supervisor, educator or attending.
A few questions to consider when writing include: Why is the issue important? What is causing the problem to persist? How might it be corrected? How is this issue important to pediatricians in training? How might it affect pediatric medicine in the future? We are looking for authors who take a stand and support it with evidence from the literature, and for articles with an "edge". A narrative thread that engages the reader and includes observations drawn from the author's clinical and professional experiences is recommended.

Points To Consider:

- The first author must be a resident, fellow, or medical student, but does not need to be a SOPT member. Collaborating authors at any career level are welcome.
- One article will be published up to every 4 months as the Monthly Feature in *Pediatrics*. High quality articles not selected for publication in *Pediatrics* will have other publication opportunities through SOPT.
- Word Limit: 1,200 words
- Reference Limit: 15 references
- Author limit: 4 authors

Specific questions may be directed to Section Editor Hannah Rosenblum, MD, here.
Historical Perspectives Monthly Feature
The historical perspectives Monthly Feature is intended to attract concise and engaging historical articles of interest to clinicians. These articles are more akin to a commentary than an original article, and cannot be expected to provide the kind of in-depth analysis expected in professional historical journals. The content may draw from original research or develop a particular insight from existing scholarship. These articles are typically qualitative, and not divided into the conventional sections appropriate for original scientific contributions. Articles are peer reviewed by professionals with both medical and historical expertise.

Consider the following points as you develop your article:

- Frame a clear question or central argument. Historical articles do not just recite chronologies or lists of persons and dates, they investigate a particular question and develop an argument, backed up by sources.
- Set your article in historical context—in its own time and place. Don't judge the past by the standards of the present. Secondary sources can be very helpful. Search for articles or books that can provide historical background. If you are not familiar with historical

scholarship, see “resources” on the Pediatric History Center page of the American Academy of Pediatrics Web site.
- Will your article be of interest to pediatricians (the main audience for Pediatrics)? Is the writing clear, organized, and easy to follow?
- Is it original? Authors who have completed longer historical projects may wish to submit a short article related to a bigger project that may attract new readers to their other scholarship.
- Are assertions in the paper accurate and supported with appropriate references? Most articles will have about 10 to 20 references. Follow the AMA Manual of Style. Specific references in longer sources may require page numbers to be noted in parentheses.

Primary sources (produced by participants or contemporaries) are preferred when possible. The goal is to provide enough information that a reader could independently confirm the assertions in the text. Secondary sources (books and reviews written by historians or physician-historians) should be cited to provide context (to frame the story in space and time) and scholarly background.

Specific questions may be directed to Section Editor Jeffrey P. Baker, MD, PhD, (contact)

Global Health Monthly Feature

The global health Monthly Feature is intended to educate and engage clinicians who might not otherwise be immersed in the global health field. Submissions should provide information or perspective on issues and initiatives of international interest, including health, nutrition, and medical care in low- and middle-income countries. Articles may be broad or specific in focus, and should include appropriate references. Please direct questions to Jay Berkelhamer, MD (contact), section editor.

COMSEP Monthly Feature

COMSEP (Council on Medical Student Education in Pediatrics) publishes articles describing initiatives and techniques that are best and/or new practices for educators who teach medical students and pediatric residents. Articles are solicited internally through COMSEP. If you have a question, please contact the current section editor Robert Dudas, MD (contact).

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Pediatrics Perspectives

Abstract length: no abstract
Article length: 1,200 words max
Author limit: Three (3) authors or fewer
Figure/table: No more than one (1) figure or table allowed

Pediatrics Perspectives are unsolicited opinion pieces that focus on issues of policy, public health, or other research and clinical topics related to infant, child, and/or adolescent health. These articles should be 1200 words maximum, be written by no more than three authors and have no more than 7 references. Pediatrics Perspectives may include one figure or one table.

The general instructions regarding submission (including cover letter, title page requirements, contributors' statement page, journal style guidance, and conflict of interest statements) also apply to Pediatrics Perspectives.

- **Download and view a sample Pediatrics Perspectives manuscript (PDF)** here**.**

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Quality Report

Abstract: 250 words or fewer (structured: see Regular Articles)
Article: 3,000 words or fewer
Supplemental content: appropriate for figures, tables, multimedia, measurement tools

Quality Reports are intended to add to our understanding of how to design and implement highly reliable systems of care that optimize the quality, safety, and value of health care delivered to children.

What is suitable to submit as a Quality Report?

- Submissions that describe sustainable, replicable initiatives evaluated using rigorous quality-improvement methods that include assessment of impact on costs will be given highest priority.
    - Submissions of initiatives undertaken by quality-improvement collaboratives or at multiple sites should include:
        - Uniform outcome assessment across sites
        - Description of site specific and overall collaborative findings
        - Key lessons learned (both positive and negative) from individual sites in relation to improvement approaches and outcomes
- Priority will be given to those manuscripts that have multiple tests of change. Pre-post studies (i.e. without multiple tests of change) will be given low priority.
- Reports of clinical trials to assess whether interventions are effective are better suited as Regular Articles.
- Reports of the development and testing of improvement-related tools to assess validity and reliability are better suited as Regular Articles.
- Pilot projects of interventions to improve quality of care may be acceptable if there are important lessons that can inform further quality-improvement efforts.
- If you are uncertain whether your manuscript is appropriate as a Quality Report, e-mail Munish Gupta, MD, MMSc (contact).

What format should authors use when submitting a Quality Report?

Authors are expected to generally follow the Standards for Quality Improvement Reporting Excellence (SQUIRE 2.0) Guidelines for reporting their quality improvement initiatives. The SQUIRE guidelines are described in detail on the SQUIRE website (www.squire-statement.org). Articles that do not adhere to SQUIRE guidelines will be considered if the authors provide a reasonable explanation as to why the improvement effort could not meet these requirements.

- The general instructions to authors regarding submission (including cover letter, title page requirements, contributors' statement page, journal style guidance, and conflict-of-interest statements) also apply to Quality Reports.
- Format the submission to follow the IMRaD (Introduction, Methods, Results, Discussion) format consistent with the rest of the journal. Authors should submit the checklist below.
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Authors should address the key aspects of SQUIRE 2.0 in their submissions, and **MUST** include this table as part of their submission (upload as a Supplemental File):

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- **Download and view a sample Quality Reports manuscript (PDF) here.**
- **Download the blank SQUIRE requirements table (DOCX) here.**

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Review Article, Systematic Reviews and Meta-Analyses

Abstract length: 250 words or fewer (structured or unstructured, depending on review type)
Article length: 4,000 words or fewer

Review Articles combine and/or summarize data from the knowledge base of a topic. Preference is given to systematic reviews and meta-analyses of clearly stated questions over traditional narrative reviews of a topic. Both types of review require an abstract; the abstract of a narrative review may be unstructured (no headings, run in a single paragraph). **See below for abstracts of systematic reviews and meta-analyses.**

The general instructions regarding submission (including cover letter, title page requirements, contributors' statement page, journal style guidance, and conflict of interest statements) also apply to Review Articles.

Systematic Reviews and Meta-Analyses

Reports of systematic reviews and meta-analyses should use the PRISMA statement (http://www.prisma-statement.org/) as a guide, and include a completed PRISMA checklist and flow diagram to accompany the main text. Blank templates of the checklist and flow diagram can be downloaded from the PRISMA Web site (http://www.prisma-statement.org/statement.htm). Structured abstracts for systematic reviews are recommended. Headings should include: Context, Objective, Data Sources, Study Selection, Data Extraction, Results, Limitations, and Conclusions (see Iverson et al[1[pp22-23]]).

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- **Download and view a sample Systematic Review/Meta-analysis manuscript (PDF)** here**.**

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Special Article

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Article length: 4,000 words or fewer
Special Articles reflect topics or issues of relevance to pediatric health care that do not conform to a traditional study format. Special Articles may address broad social and ethical issues, scientific methodology, or other scholarly topics, and may include reports from consensus committees and working groups. These articles should not include specific guidelines or recommendations for practice. Guidelines and recommendations from groups outside of the AAP must be approved through the AAP and may be published at the discretion of the AAP in the Academy's dedicated section of the journal (see below).

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State-of-the-Art Review Article

Abstract length: 250 words or fewer (unstructured: no headings, run in a single paragraph)
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State-of-the-Art Review Articles provide a comprehensive and scholarly overview of an important clinical subject with a principle focus on developments in the past 5 years. State-of-the-Art Articles are usually invited. If you are interested in submitting a State-of-the-Art Review, please email Associate Editor Dr. Steven Zeichner (contact) and copy Publications Editor Mark Plemmons (contact).
The general instructions regarding submission (including cover letter, title page requirements, contributors' statement page, journal style guidance, and conflict of interest statements) also apply to State-of-the-Art Reviews.

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The cover letter serves to assure the editors that the article and the authors meet the conditions of publication. A brief paragraph that provides any additional information that may be useful to the editors is welcome, but keep in mind that the need for a long cover letter may indicate that the article does not speak for itself. Reviewers will not see the cover letter; cover letters are not a Title Page.

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- That all authors are responsible for reported research.
- That all authors have participated in the concept and design, analysis and interpretation of data, and drafting or revising of the manuscript, and that they have approved the manuscript as submitted.

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2. Sign in and select "Author Center."
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5. Click "Submit".

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2. Recent comments also located on the home page in the "Recent Comments" box.

How to Cite a Comment

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Quartermain, Michael D., Prenatal Diagnosis Data [comment], Pediatrics (October 27, 2015), https://pediatrics.aappublications.org/content/136/2/e378/tab-e-letters#prenatal-diagnosis-data (accessed November 15, 2019).
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All Letters to the Editor must first be submitted as online comments (and must conform to comment requirements). Selected comments may then be chosen for publication in the indexed edition of *Pediatrics* as "Letters to the Editor." The editors may choose to abridge and edit a comment prior to publication as a Letter to the Editor in *Pediatrics* without notifying or seeking approval from the author. Only these selected responses will be cited in MedLine.
At the time of provisional acceptance, the comment author will receive instructions for submitting an online copyright form. No comment will be scheduled for an issue's Letters to the Editor section and move onto production until the copyright form is complete.

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Errata

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