CLINICAL FEATURES AND HAEMATOLOGICAL INDICES OF BACTERIAL INFECTIONS IN YOUNG INFANTS

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ABSTRACT

The clinical features and haematologic indices of 100 young infants aged 3 months and below, admitted with suspected bacterial infections, were analysed. Fever, lethargy, hepatomegaly, poor feeding and irritability were the commonest features for suspecting a bacterial infection in these infants. However, the features significantly associated with bacterial infections were respiratory distress and cyanosis. Of the haematologic indices commonly associated with bacterial infections, only C-reactive protein and erythrocyte sedimentation rate were significantly predictive compared to leukocyte counts, absolute neutrophil counts and nitro-blue tetrazolium test. When used in combination, a raised C-reactive protein with erythrocyte sedimentation rate, a raised erythrocyte sedmentation rate with abnormal leukocyte counts and a raised C-reactive protein with abnormal leukocyte counts were significantly associated with bacterial infections.

Keywords: bacterial infections, young infants, clinical features, haematologic indices.

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INTRODUCTION

Bacterial sepsis in the young infant can be a life-threatening condition, especially in the neonates. The incidence of neonatal septicaemia ranges from 1 to 10 per 1000 livebirths[1-3] and despite the great strides made in diagnostic capabilities, the incidence do not appear to have decreased significantly over the last two decades[3]. The overall mortality ranges from 12% to 90%[1-7] depending on numerous factors including the presence of perinatal conditions that predispose to infection, maturity and physiological status of the infant at time of presentation as well as early diagnosis and initiation of appropriate antibiotics. The discovery and judicious use of antibiotics had made a significant difference in the mortality rates, from 90% prior to 1936 to the present day incidence of 12% to 20%[4,7]. What is disturbing however, is the fact that although the mortality rates had shown a decline, the incidence of moderate to severe handicaps as a direct result of septicaemia in the survivors, have remained unchanged at about 20%[5,7]. 60% of these handicapped children had osteomyelitis or meningitis[5,7].

What is eminently a treatable condition is often complicated by the fact that most features of sepsis in the young infants are vague and non-specific[8-12]. It has been stressed often enough that septic infants need to be identified early for initiation of antibiotic therapy. However, often overlooked but equally important is the identification of the nonseptic infant. So much has been said and published about the mortality and morbidity of this condition that most young infants presenting with fever would be admitted and subjected to a complete "septic workup" and started on intravenous antibiotics until culture results are available. This raises the problem of subjecting such infants to unnecessarily prolonged and expensive hospitalisation, invasive procedures as well as the possible emergence of antibiotic resistant strains of bacteria[13-17]. Increasing objections are being raised about the use of antibiotics as a "prophylaxis" in such situations[13,15,16].

This study was formulated to identify the clinical features and haematologic indices of bacterial infection amongst young infants and to determine retrospectively the findings significantly associated with positive bacterial cultures. As our main aim was to identify the infant requiring antibiotics an admission, this group of infants would include not only those with positive blood cultures but positive urine. CSF and sputum cultures as well. The study was limited to those less than 3 months of age as from past experience, it is this group of patients whose clinical features of a bacterial infection are vague and are thus most likely to be subjected to invasive laboratory investigations and antibiotics.

MATERIAL AND METHODS

During the period July 1989 to February 1991, 100 infants ≤3 months of age were evaluated for possible bacterial infections upon admission to the Paediatric Department, Tan Tock Seng Hospital. These infants were suspected on clinical grounds to have a bacterial infection and a full "septic workup" and intravenous antibiotics were being contemplated. A detailed history and clinical examination was subsequently performed by the authors, who looked for the following features in every patient:

General features	: Fever or Hypothermia
Cardiovascular system	: Mottled skin, hypo or hypertension, cold clammy peripheries, arrhythmias
Respiratory system	: Cyanosis, grunting, respiratory distress, apnea.
Central Nervous system	: Lethargy, irritability, hypotonia, seizures.
Gastrointestinal system	: Poor feeding, diarrhoea, hepatosplenomegaly, abdominal distension
Skin	: Pustules, umbilical sepsis, bleeding tendency.

Laboratory tests done include the following:

1) Total white blood cell count
2) Absolute neutrophil count
3) Platelet count
4) Immature to Total Neutrophil ratio (I/T ratio)
5) Nitroblue Tetrazolium test (NBT)
6) C-reactive protein (CRP)

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7) Erythrocyte Sedimentation Rate (ESR)
8) Chest X-ray (CXR)
Sputum culture (via nasopharyngeal aspiration) was done if CXR showed any infective changes.
9) Blood culture (aerobic) x 2
10) Urine culture x 2
11) CSF FEME and culture (only when meningitis was suspected on clinical grounds)
12) Culture from skin pustules or umbilicus when indicated.

The infant was then started on intravenous antibiotics pending these results. The absolute neutrophil count, platelet count and I/T ratio were read by the same senior laboratory technician to ensure consistency. A total of 5cc of venous blood was taken with each evaluation.

Interpretation of results

1) Total white cell counts

Age-adjusted normal values were used. Total leukocyte count as measured by a Coulter Counter was abnormal if less than 5000/mm^3[18,19]. Raised counts were abnormal if

a) ≥ 25000/mm^3 at birth
b) ≥ 30000/mm^3 from 12 hours to 24 hours of age and
c) ≥ 21000/mm^3 from Day 2 onwards.

2) Absolute Neutrophil counts

Age-adjusted values based on reference ranges published by Manroe[19] were used. Generally the following were considered abnormal:

a) ≥ 14400/mm^3 at 12hrs of life
b) ≥ 12600/mm^3 at Day 1
c) ≥ 9000/mm^3 at Day 2
d) ≥ 7000/mm^3 at Day 3
e) ≥ 5400/mm^3 from Day 5 onwards, and
f) ≤ 1000/mm^3 at any age

3) Absolute Platelet counts

A platelet count <150,000/mm^3 was considered abnormal[20].

4) Immature to Total Neutrophil ratio

Neutrophilic granulocytes in the blood smear were divided into nonsegmented (immature) and segmented (mature) types. A cell was considered to be segmented if its nucleus was distinctly segmented into two or more lobes connected by a thin filament whose width was less than one-third the maximum diameter of the lobes[19,21]. Cells with no lobulation or those in which apparent lobes were joined by a thick band of chromatin were referred to as non-segmented or immature neutrophils[19,21].

An I/T ratio of > 0.2 was considered abnormal[22].

5) Nitroblue Tetrazolium Test

0.25mls of venous blood was collected in a lithium-heparin micro-tube. 0.1ml of this blood was then added to 0.1ml of NBT solution (made with equal parts of 0.2% NBT and 0.15M phosphate-buttered saline solution). Incubation was carried out at 37°C for 25 minutes. The blood is then smeared and stained with Wright's stain. The slides were examined microscopically under oil immersion and 100 neutrophils were counted. Those with black formazan deposits were counted as positive. The percentages of positive NBT neutrophils were then reported.

A score of > 12% was taken as abnormal[23].

6) C-reactive protein

CRP was measured using the latex reagent method. A level of > 10mg/l was considered abnormal.

7) Erythrocyte Sedimentation Rate

ESR was measured using 2ml of venous blood in sodium citrate solution. (macro-ESR method of Wintrobe).

A value > 15mm for the first hour was considered abnormal[22,24,25].

8) Designation of Infection status

Infants with positive bacterial cultures of a pathogenic organism from the blood, CSF, urine, sputum, pustules or umbilicus were designated as having proven bacterial infection. Non-pathogenic organisms considered as contaminants include Bacillus subtilis, diptheroids, lactobacillus and alpha-streptococcus. Two cultures of blood or urine with the same organism must be present before these are considered positive. Patients known to be already on antibiotics when the evaluations were performed were excluded from the study.

Analysis of Data

The infants were assigned to two groups (infected or non-infected) depending on positive or negative cultures. The sensitivity and positive predictive accuracy for each clinical feature and haematologic index were calculated[26]. Special emphasis was placed on sensitivity ("if infection is present, is the clinical feature or laboratory test abnormal?") and positive predictive accuracy ("if clinical feature is present or test abnormal, what is the probability of infection being present?")[26]. In clinical situations, the positive predictive accuracy would be more appropriate.

Chi-squared test with Yates correction was used for statistical analysis.

RESULTS

During the study period 100 infants underwent the above evaluations. There was no mortality.

The mean age group was 4.6wks ± 3.06 ($\bar{x}$ ± S.D.) There were 60 males and 40 females.

Thirty of 100 (30%) workups yielded positive bacterial cultures. Twenty of these patients were males (66%).

There were 6 positive blood cultures out of 100 evaluations. Five of these patients also yielded positive culture results from other sites indicating the probable origin of the bacteremia. (One patient had group B streptococcus meningitis with a positive CSF culture, one had Salmonella Group D enterocolitis with positive stool culture, one had *Staph. aureus* cultured from multiple skin pustules, one had *staph aureus* from pus obtained from drainage of the ethmoid sinus and one had *E.Coli* isolated from urine cultures). The last patient had *E.Coli* septicaemia but stool and urine cultures were negative.

There were 11 positive sputum cultures out of 27 evaluations (27 out of 100 chest X-rays done were suspicious of infection). Twelve urine cultures were positive out of 100 evaluations. One CSF culture was positive out of 42 lumbar punctures done. One patient had a positive culture from eye swab and 1 patient had a positive culture from umbilical swab.

Slightly more than half(16) of these infants with positive bacterial cultures were in the neonatal age group. Six neonates had bacterial chest infections, 5 had urinary tract infection, 1

Table I – Bacterial isolates from 100 patients evaluated for infection

Source	Bacteria	No.
Urine	E. Coli	8
	Klebsiella	4
Sputum	Staph aureus	7
	MRSA	3
	Klebsiella	1
CSF	Gp B Streptococcus	1
Stool	Salmonella Gp D	1
Skin Pustule	Staph. aureus	1
Eye swab	Staph. aureus	1
Umbilical swab	Staph. aureus	1
Ethmoid sinus	Staph. aureus	1
Blood	E. Coli	2
	Staph. aureus	2
	Gp B Streptococcus	1
	Salmonella gp D	1

had meningitis, 1 had impetigo, 1 had eye sepsis, 1 had umbilical sepsis, and 1 had bacteremia of unknown origin.

The bacteria isolated from those with positive cultures are shown in Table I. Staph aureus was the commonest bacteria isolated, followed by E.Coli.

The 10 most common clinical features that lead to a suspicion of possible bacterial infections in the infants are listed in Table II.

Table II – 10 most common clinical features that lead to suspicion of a bacterial infection in 100 patients

Clinical features	No
Fever	85
Lethargy	44
Hepatomegaly	39
Poor feeding	35
Irritability	30
Splenomegaly	23
Skin mottling	17
Diarrhoea	15
Respiratory distress	12
Hypotonia	12

The most common clinical features of bacterial infections in the 100 patients evaluated are shown in Table III.

The commonest reasons for suspecting infection were fever, lethargy, hepatomegaly and poor feeding. Although these features are associated with comparatively high sensitivities, their positive predictive accuracies are low (ie they are likely to be found equally commonly in patients without bacterial infections). We found that only respiratory distress and cyanosis were significantly associated with bacterial infections (Table III).

Among the haematological tests evaluated, CRP and ESR both had high sensitivities and positive predictive accuracies. They were significantly associated with bacterial infections. Of note is the high specificity and negative predictive accuracies of all the tests (ie. if the tests are negative, it is highly unlikely for the patient to have a bacterial infection). The evaluations of the haematologic tests are shown in Table IV.

Combinations of the single tests were then analysed. As CRP, ESR, WBC counts and neutrophils counts gave the highest positive predictive accuracies, the results from combining any two of these tests were analysed (Table V).

Table III – Most common clinical features of bacterial infections in young infants.
Positive evaluations/Total evaluations : 30/100 (30%)

Clinical	Infected	Non-Infected	Positive predictive accuracy(%)	Sensitivity (%)	P Value
Respiratory distress	7	5	58	23	<0.01
Cyanosis	6	5	55	20	<0.05
Grunting	5	7	42	17	NS
Splenomegaly	9	14	39	30	NS
Hepatomegaly	15	24	38	50	NS
Fits	4	7	36	13	NS
Mottled skin	6	11	35	20	NS
Hypotonia	4	8	33	13	NS
Diarrhoea	5	10	33	17	NS
Fever	28	57	33	93	NS
Lethargy	13	31	30	43	NS
Poor feeding	10	25	29	33	NS
Irritability	7	23	23	23	NS
Vomiting	2	10	17	7	NS

The combinations of CRP with ESR, ESR with WBC counts and CRP with WBC counts were significantly associated with bacterial infections.

DISCUSSION

The recognition of the young infant with a bacterial infection remains a challenge primarily because the clinical features are so non-specific. Commonly perceived features of bacterial infection like fever, lethargy, poor feeding and irritability remain the starting point for the recognition of a septic baby in many published septic evaluations[9,11,12,22,25]. However, it is now clear that these features are least likely to be associated with positive bacterial cultures[9] and we would be subjecting such infants to unnecessary tests and antibiotics.

In our patients, the commonest clinical features that lead to a suspicion of a bacterial infection were fever, lethargy,

Table IV – Haematologic findings in young infants with bacterial infections
Positive evaluations/Total evaluation : 30/100 (30%)

	Total +ve tests	+ve test with -ve cultures	+ve test with -ve cultures	+ve predictive accuracy (%)	sensitivity (%)	specificity (%)	-ve predictive accuracy (%)	P Value
Abnormal WBC counts	21	8	13	38	26.7	81.4	72	NS
Absolute neutrophil counts	55	16	39	29	53	44	68	NS
Abnormal platelet counts	5	1	4	20	3.3	94	69	NS
Raised I/T ratio	15	4	11	26.7	13	84	69.4	NS
Raised NBT	13	4	9	30.8	13.3	87.1	70.1	NS
Raised CRP	66	25	41	37.9	83.3	41.4	85.3	<0.01
Raised ESR	54	21	33	38.9	70	52.9	80.4	<0.05

(Sensitivity : +ve culture with +ve tests, specificity : -ve cultures with -ve test
+ve predictive accuracy : +ve test with +ve culture.
-ve predictive accuracy : -ve test with -ve culture).

Table V – Results of combination of some haematologic tests

	Total +ve tests	+ve test with +ve cultures	+ve predictive accuracy (%)	sensitivity (%)	specificity (%)	-ve predictive accuracy (%)	P Value
CRP and ESR	43	18	42	60	64	78	<0.05
CRP and WBC counts	16	8	50	27	89	84	<0.05
CRP and neutrophil counts	39	14	36	47	64	74	NS
ESR and WBC counts	15	8	53	27	90	74	<0.05
ESR and neutrophil counts	33	13	39	43	71	75	NS
WBC counts and neutrophil counts	19	7	37	23	83	72	NS

poor feeding and hepatomegaly. However, these are all associated with low positive predictive accuracy and are not significantly associated with positive bacterial cultures. Features of respiratory distress, cyanosis, grunting and hepatosplenomegaly are more likely to be associated with infections as they have a higher positive predictive accuracy. Respiratory distress and cyanosis were significantly associated with positive cultures. Other authors have found varying combinations of clinical features to be significantly associated with infections. Nyhan[4] found fever, jaundice and hepatomegaly to be significant; Follner[12] found skin mottling and respiratory distress to be good indicators of septicaemia and in Spector's[9] patients, a significant number had poor peripheral circulation, tachycardia and arrhythmias. Our finding of respiratory distress and grunting to be significantly associated with bacterial infections appear to be of little clinical value however, for it would take a brave paediatrician not to start antibiotics on a suspected septic infant who is cyanotic and in respiratory distress. Thus the challenge of identifying the few septic infants amongst the many normal ones who present with common features of fever, lethargy and poor feeding remains.

The multitude of published data on the many laboratory tests and their varying combinations into a "scoring" method is clear testimony to the fact that no single test is at present sufficiently accurate or reliable in identifying an infected infant[9,11,12,22-25,27-48]. The accuracy of such tests vary according to the laboratory technique used, observer error as well as the selection of patients. In this study therefore, we have attempted to assess the usefulness of some of these tests using our own hospital laboratory techniques and technicians.

Since the redefinition of the limits of normality of absolute white cell counts according to age, there has been renewed interest in the use of white cell counts in diagnosing bacterial infections in young infants[19]. It has been demonstrated that mild nonfatal bacterial infections often show no haematologic abnormalities, while more severe nonfatal bacterial sepsis may demonstrate an increase in white cell counts, often due to a rise in mature and immature neutrophils [21,32]. On the other hand, very severe and fatal bacterial infections are often characterised by leukopenia, neutropenia and thrombocytopenia[19,32]. Analysis of bone marrow histopathology showed that these lowered values are most likely the result of consumptive or sequestration mechanisms as suggested by Zipursky and Christensen[31,49] rather than by bone marrow suppression. In this study, the leukocyte count appeared to have a higher positive predictive accuracy when compared to the rest but is however not significantly associated with bacterial infections. Its high specificity and negative predictive accuracy makes it useful for excluding the presence of infection in the face of normal WBC counts.

Similarly, although much has been said about the association of a raised neutrophil count with bacterial infections[32], there are some who have failed to significantly demonstrate such an association in young infants[9,35,46,47]. On the contrary, many neonates with severe bacterial infection especially those who eventually succumbed have neutropenia instead[9,46,48]. This is likely to be due to a depleted neutrophil storage pool and is significantly associated with increased mortality[38,49]. There were no patients with neutropenia in this study. In our patients, the neutrophil count is associated with a low positive predictive accuracy and not significantly associated with infections. As a single test, it is unlikely to be useful in predicting infection.

A shift to the left in differential white counts with a raised immature neutrophil count has been documented in patients with bacterial infections[37,38]. This had led to the use of the I/T ratio as a pointer towards bacterial infections. This ratio is still believed by many to be the single most helpful test available[9,12,22,25,32,35,38,46,48]. We however could not demonstrate an association between a raised I/T ratio and bacterial infection. In fact, in our series, the I/T ratio was associated with a low sensitivity and positive predictive accuracy compared to the other tests. Some authors have found this to be true in their studies as well[34,47]. The I/T ratio is highly operator dependent and accuracy relies to a large extent on correct identification of the immature neutrophils by the laboratory technician. This ratio is also believed to be less sensitive after the first week of life[25].

The relationship between thrombocytopenia and sepsis was established by Corrigan[20] in infants and later by Zipursky[31] in prematures. It has also been reported that fatal bacterial infections are often associated with adrenal haemorrhage and thrombocytopenia with megakaryocytopenia[32]. We however obtained a very poor yield when using thrombocytopenia as an indicator of bacterial infections. This may be because viral infections are often associated with low platelet counts as well and this probably accounts for the poor predictive accuracy reported by other authors too[9,32,34,35]. The exact cause of thrombocytopenia in sepsis is as yet unclear although suggestions ranging from endotoxin destruction, increased utilisation and intravascular coagulation have been postulated[31].

The nitroblue tetrazolium test is a non-specific test of neutrophil membrane stimulation and was introduced by Park[50] as a rapid diagnostic test for systemic bacterial infection. However subsequent reports have demonstrated that the test is subject to many possible laboratory errors and results vary so widely between different laboratories that many have come to conclude that it is not a useful diagnostic test[23]. Recent im-

provements and streamlining of the tests have however shown that it can be a useful test for bacterial infection[23,47,48]. Using our own laboratory method, we found that NBT by itself is not sufficiently accurate as a diagnostic test.

The erythrocyte sedimentation rate is a nonspecific test of tissue damage. It is known to be elevated in infections as well as collagenosis, malignancies and infarctions. Although it is nonspecific, since the test can be performed using capillary blood, it would appear ideally suited for use in an infant. Studies have shown that ESR can be used as a diagnostic aid[11,22,24]. In this paper, ESR is raised in more than half of the patients evaluated and in 70% of those with a positive culture. It is significantly associated wth a bacterial infection and appears useful as a single test for infections. When used in combination with an abnormal leukocyte count, it is also predictive of a bacterial infection.

The presence of C-reactive protein in human serum has long been used as a sensitive indicator of an infection or inflammatory process and it has been shown that the levels are directly proportional to the severity of the infection[28]. It has been initially presumed to be absent in the newborn[28] but with more accurate assaying methods, a range of CRP in healthy infants have been established[29,41,51]. Sensitivity of the test is to a large extent dependent on the assaying techniques used. The latex agglutination method is the commonest method used as it is a simple and rapid test. Most published papers using CRP as a screening test utilises this method[11,22,25,47]. More accurate methods described but unavailable in our laboratory include laser nephelometry[27], enzyme immunoassay method[27], ligand-binding radiometric monoclonal antibody immunoassay[41] and radial immuno diffusion assay methods[51]. Normal ranges vary according to the technique used.

Our laboratory uses the latex agglutination method and a normal range of 0-10 mg/l is given. Using this method, we found that a raised CRP is significantly associated with bacterial infections and appears to be a useful single diagnostic test. CRP is also a good prognostic indicator. A high level followed by a rapid decline is associated with a good outcome[30,39]. Early initiation of appropriate antibiotics can blunt or prevent a CRP response and this has been advocated as a possible method of monitoring the effectiveness of antibiotic therapy[41]. However, the latex agglutination method which depends on an experienced technician to determine the end-point of the reaction is not sensitive enough to be used for serial monitoring and in this context, we do not think that we can use the CRP to monitor the progress of our patients. Nevertheless, as it provides a reliable distinction between normal and raised levels, we advocate that CRP be used in conjunction with ESR as a diagnostic test for bacterial infections in young infants.

In this paper we have attempted to single out those useful tests in identifying an infected infant. Of the single tests, only CRP and ESR were significantly associated with positive cultures. When used in combination, CRP with ESR, ESR with WBC counts and CRP with WBC counts appear to be useful. Other laboratory tests have also been described as useful by various authors eg. IgM levels[43-45], orosomucoid (alpha 1 - acid glycoprotein) levels[30,39,40], acridine orange-stained buffy coat smears[42,47], degenerative changes (eg vacoulisation and toxic granulation) within the neutrophils[31,34], buffy coat smear for leukocytic intracellular organisms[33] and serum haptoglobin levels[11,22,25]. These tests however were not considered in our study because they are not readily available in our laboratory and results are not rapidly available to be of much predictive value in the early phases of an infant's admission to hospital.

What is enlightening also is the high specificity and negative predictive accuracy of most of the tests. This implies that a patient with negative tests is highly unlikely to have a bacterial infection and antibiotics may be withheld till availability of culture results.

Before the availability of culture results, we empirically start our patients on intravenous ampicillin and gentamicin. Ampicillin would be useful against the gram positive organisms especially the cocci (eg Streptococci and Meningococci). It is also effective against some gram negative organisms eg Proteus, Haemophilus influenza, E.Coli and certain strains of Klebsiella and Salmonella. Gentamicin is especially helpful against gram negative organisms like E. Coli. Judging from the pathogens isolated in this study, this antibiotic regime would have been adequate against two-thirds of the cases (ie E.Coli, Klebsiella, Gp B streptococcus and Salmonella). What appears disturbing is the large number of cases with Staph aureus infection (one-third), the majority due to chest infection. However, the large number of cases of Staph aureus isolated from sputum cultures could be falsely high as the majority of the sputum culture were obtained via nasopharyngeal aspiration. This could have lead to contamination from Staph aureus in the nose and throat. Nevertheless, we suggest that Cloxacillin be added to the regime of ampicillin and gentamicin in those septic infants with suspected chest infection.

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