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Receiver operating characteristic curves for comparison of serial neutrophil band forms and C reactive protein in neonates at risk of infection

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Abstract

The performance of indirect indices of infection in the newborn vary because of differences in techniques, including diagnostic cut off levels. We have compared serial neutrophil band cell counts with C reactive protein measured by rate nephelometry. The 'gold standard' was a positive culture and the performance of the tests was compared by the technique of receiver operating characteristics (ROC) as well as sensitivity and specificity.

A total of 172 septic screens were performed in 56 patients. The operational diagnostic cut off values were: C reactive protein >8 mg/l, immature:total neutrophil ratio (I:T ratio) >0·2, and band count >5%. Compared with the sensitivity of C reactive protein (71%), I:T ratio (34%) was significantly different but band count (69%) was not. The specificity of C reactive protein (72%) was better than band count (39%) but no better than I:T ratio (73%). ROC curves were constructed for all possible diagnostic cut off values of the tests and superior performance was demonstrated for C reactive protein compared with band count and I:T ratio.

We conclude that C reactive protein is a useful early indicator of infection in neonates and that ROC curves permit comprehensive and graphic comparison between tests and the calculation of optimal diagnostic cut off values.

Early clinical diagnosis of neonatal infection is difficult because of the non-specific presentation of infection, and a delay in diagnosis may be associated with increased morbidity and mortality. Early laboratory indicators of infection are therefore required to facilitate early diagnosis.[1–10] Infection activates the acute phase response which may be detected by alterations in the peripheral blood neutrophils[1–3] and an increase in serum proteins such as C reactive protein.[5] Although such changes are non-specific, they are thought to give indirect evidence of infection.[3–8]

Single determination of neutrophil band forms and other indices of infection has been used for early diagnosis of infection while the results of blood cultures are awaited,[3 5–7 10] but wide variation exists in the reported performance of these tests. Combinations of tests have been used to improve the test performance of single determinations,[5 10 11] while serial determination of C reactive protein has also been utilised to improve sensitivity by detecting changes in C reactive protein rather than specific diagnostic cut off points.[12] Serial C reactive protein has also been used for monitoring of antibiotic treatment and to detect early recurrence of infection.[13 14]

Standard non-parametric statistical comparisons of test performances such as χ^2 and McNemar tests may be complemented by the calculation of receiver operating characteristics (ROC),[15] permitting direct comparison of tests over *all* diagnostic cut offs. We have therefore used this technique as well as standard statistical methods to compare the performance of paired serial C reactive protein concentrations and neutrophil band counts in neonates with suspected infection.

Subjects and method

All babies admitted to the unit for either suspected infection or management of prematurity, low birth weight, respiratory distress syndrome, and asphyxia neonatorum were studied over a three month period. Daily samples of 0·5 ml of blood, collected in EDTA, were drawn from arterial/umbilical arterial lines or from capillary blood for routine full blood count. This sample was used for the differential count including band forms and the remaining plasma was used for determination of C reactive protein. A septic screen (culture of blood, cerebrospinal fluid, urine, and endotracheal secretions) was performed when indicated and processed according to standard microbiological procedures. Infection was suspected on the basis of at least one of the following criteria. (1) Clinical indication: including prolonged rupture of membranes, respiratory distress syndrome, outborn, apnoea, lethargy, irritability, temperature instability, non-specific abdominal distension, and unexplained metabolic acidosis. (2) Neutrophil band count of >5% of total white cell count (approximately equivalent to immature neutrophil:total neutrophil (I:T) ratio of 0·11 (95% confidence interval 0·07 to 0·15, unpublished data). (3) C reactive protein concentration of >8 mg/l. These values constitute the operational diagnostic cut off values for the tests, above which the test is considered positive for infection. The clinician responsible for the care of the patient at the time of suspected infection determined the need for culture and treatment.

C reactive protein was measured by rate nephelometry using a Beckman Array System

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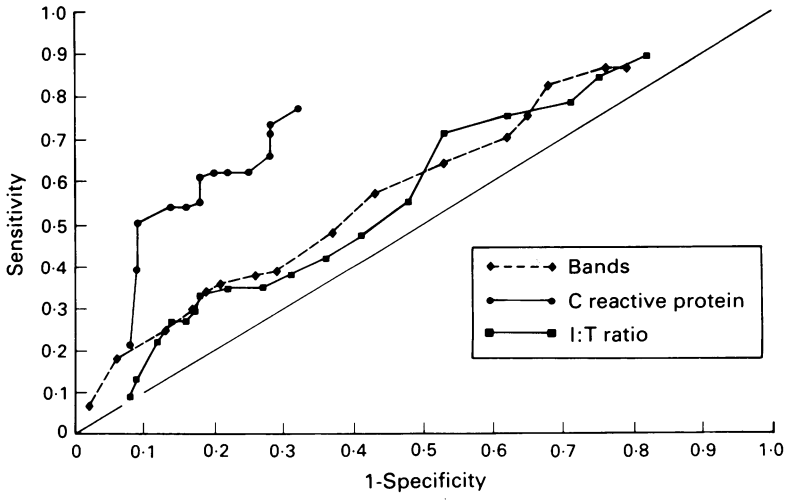
protein analyser (Beckman Instruments, C reactive protein reagent kit 449760). An aliquot of 50 µl plasma was diluted with 250 µl of Beckman buffer and centrifuged to remove immune complexes and limit turbidometric interference. The lower detection limit used was 6 mg/l because of increased interference from other proteins below concentrations of 4–6 mg/l. The within-run coefficient of variation of the test was 3–5% and between-run coefficient of variation was <5%. The Beckman assay has been compared with electroimmunodiffusion and radial immunodiffusion assays and correlation coefficients of 0·986 and 0·993 respectively were obtained.[16]

Blood counts were estimated by Coulter counter in the routine paediatric haematology laboratory and neutrophil band counts were determined by duty technicians in accordance with accepted criteria for the detection of band forms,[5] but neutrophil precursors before band forms were recorded separately.

When considering the study size a false positive rate of 60% for band forms was estimated and a 50% reduction in false positives was considered clinically worthwhile. Forty nine pairs of C reactive protein concentrations and band counts were required to detect such a difference at the 0·05 level of significance (two sided) and with 90% confidence.[17] Three methods of comparison of test performance were used. (1) Comparison of the binomial proportions (sensitivity, specificity, and positive predictive accuracy) was by the χ^2 test with Yates's continuity corrrection and (2) the McNemar test was used to compare the ability of the test to predict the culture result. These two methods compared the tests at single diagnostic cut off levels. (3) ROC of the infection indices.[15] This technique allows a comparison to be made of a test's ability to discriminate between two populations regardless of diagnostic cut off levels selected. The ROC curve is constructed by plotting the test sensitivity (equivalent to the true positive rate) on the y axis and 1-specificity (equivalent to the false positive rate) on the x axis, for all possible cut off values of the diagnostic test (see figure). The diagonal line represents a test that produces false positive results (x axis) at the same rate as true positive results (y axis). A good diagnostic test would be represented by a ROC curve in the upper left hand triangle as close as possible to the 'north west' corner.



ROC curves of infection indices. 1-Specificity is equal to the false positive rate.

Table 1 Patient details

No of patients	56
Median gestation (weeks)	31
Range	25–40
Median birth weight (g)	1448
Range	693–3862
M:F	33:23
No with respiratory distress syndrome	
On IPPV*	43
On oxygen	7
No on total parenteral nutrition	43
No of deaths	7
Median day of screen	13
Range	1–131
Median No of screens	5
Range	1–11

*IPPV=intermittent positive pressure ventilation.

Results

A total of 172 septic screens were performed on 56 patients. The median gestational age (range) of the 56 patients was 31 weeks (25–40) and median weight (range) 1448 g (693–3862). Details of the patients are shown in table 1.

Cultures were taken between one and 11 times (median 5) from each patient. Of the 172 cultures, 28 were performed on admission on day 1 and of these 2/28 (7%) were positive (one *Escherichia coli* and one *Staphylococcus epidermidis*). Fifty four (37·5%) of the remaining 144 cultures were positive giving an overall positive culture rate of 56/172 (32·5%). The frequency of organisms cultured is shown in table 2 and the infection indices of positive cultures are shown in table 3.

The culture result was regarded as the 'gold standard' against which to compare the performance of the acute phase indices. The sensitivity (proportion of positive cultures detected by the test), specificity (proportion of negative cultures correctly identified by the test), and efficiency (proportion of all culture results correctly determined by the test – that is, the sum of sensitivity and specificity[18]), were calculated. C reactive protein had a sensitivity of 71·4% and specificity of 72·4% as compared with percentage band count with sensitivity of 69·6% and specificity of 38·8%. The sensitivity of the I:T ratio was 34% and specificity of 73%.

Table 2 Culture results

Organism	*No of cultures*
Staphylococcus epidermidis	41
Staphylococcus aureus	4
Escherichia coli	2
Klebsiella pneumoniae	2
Pseudomonas aeruginosa	1
α Haemolytic streptococcus	1
Bacillus species	1
Neisseria meningitidis	1
Candida albicans	3
Total No of positive cultures	56
Total No of negative cultures	116
Total No of cultures	172

Table 3 Acute phase indices for positive cultures (n=56)

Organism	*Initial C reactive protein (mg/l)*	*Peak C reactive protein (mg/l)*	*Band count*	*I:T ratio*
α Haemolytic streptococcus	30	62	9	0·16
Staphylococcus aureus	14	60	21	—
Staphylococcus epidermidis	31	31	7	0·12
Staphylococcus epidermidis	75	84	7	0·10
Escherichia coli	7	82	31	0·57
Klebsiella species	251	251	14	0·65
Staphylococcus epidermidis	6	6	8	0·14
Staphylococcus epidermidis	6	43	8	0·15
Staphylococcus epidermidis	6	30	1	0·01
Staphylococcus aureus	136	227	20	0·40
*Neisseria meningitidis**	88	120	35	3·18
Escherichia coli	8	115	26	1·18
Staphylococcus epidermidis	18	58	9	0·12
Staphylococcus epidermidis	39	92	15	0·23
Pseudomonas aeruginosa	13	181	4	0·17
Staphylococcus epidermidis	22	84	9	0·11
Staphylococcus epidermidis	14	115	13	0·20
Staphylococcus epidermidis	23	66	4	0·04
Staphylococcus epidermidis	39	70	27	0·41
Staphylococcus epidermidis	70	70	0	0·00
Staphylococcus epidermidis	107	119	0	0·00
Staphylococcus epidermidis	6	6	0	0·00
Staphylococcus epidermidis	6	6	17	0·38
Staphylococcus epidermidis	50	91	9	0·11
Staphylococcus epidermidis	8	66	5	0·07
Staphylococcus epidermidis	10	10	6	0·12
Candida albicans	32	44	26	0·49
Staphylococcus epidermidis	35	53	33	0·82
Staphylococcus aureus	27	92	24	0·46
Staphylococcus epidermidis	9	12	8	0·12
Staphylococcus epidermidis	79	79	16	0·26
Staphylococcus epidermidis	6	6	54	3·18
Bacillus species	6	60	17	0·34
Candida albicans	65	93	11	0·18
Staphylococcus epidermidis	26	64	5	0·07
Staphylococcus epidermidis	42	42	5	0·05
Staphylococcus epidermidis	7	12	6	0·10
Staphylococcus epidermidis	19	62	14	0·30
Staphylococcus epidermidis	6	—	1	0·04
Staphylococcus epidermidis	9	10	1	0·02
Staphylococcus epidermidis	14	145	0	0·00
Staphylococcus epidermidis	175	190	7	0·12
Staphylococcus epidermidis	6	6	5	0·16
Staphylococcus epidermidis	6	6	3	0·04
Staphylococcus epidermidis	6	6	8	0·11
Candida albicans	32	112	15	0·26
Staphylococcus epidermidis	24	27	7	0·10
Staphylococcus epidermidis	45	45	9	0·16
Staphylococcus epidermidis	6	6	4	0·11
Staphylococcus epidermidis	6	6	1	0·02
Staphylococcus epidermidis	175	190	7	0·12
Staphylococcus epidermidis	10	42	10	0·24
Staphylococcus epidermidis	15	15	6	0·12
Staphylococcus epidermidis	148	148	8	0·12
Staphylococcus epidermidis	39	70	27	0·41
Staphylococcus aureus	61	116	21	0.52

*In the cerebrospinal fluid.

The performance characteristics are shown in table 4. As the tests were paired, the McNemar test was used to test the hypothesis that no difference existed between the ability of the tests to discriminate between infected and non-infected populations. C reactive protein was significantly different from both bands (χ^2=16·7, p<0·0001) and I:T ratio (χ^2=5·8, p<0·05). C reactive protein was more efficient than bands and I:T ratio in all three postnatal period divisions but this only achieved statistical significance (p<0·05) when compared overall with neutrophil bands.

The ROC curves are shown in the figure. The C reactive protein curve is to the left of both bands and I:T ratio indicating superior performance at all cut off values. Statistical comparison at single points on the ROC curves of the tests was possible by χ^2 for paired samples by selecting arbitrary points on each curve with equivalent sensitivity. When a sensitivity point of 0·71 for C reactive protein was compared with the closest equivalent band count sensitivity of 0·70 a significant difference was present (χ^2=18·55, p<0·001) and similarly for C reactive protein (0·71) and I:T ratio (0·71) (χ^2=10·78, p<0·01). When equivalent false positive rates (1-specificity) were compared for C reactive protein (0·28) and bands (0·29) (χ^2=5·31, p<0·05) and for C reactive protein (0·32) and I:T ratio (0·31) (χ^2=5·014, p<0·05) significant differences existed confirming the superior performance of C reactive protein as implied by the position of the C reactive protein ROC curve. If a complete C reactive protein curve could have been plotted then the area under the curves could have been compared to quantitate statistically the performance of the test over the entire range of possible cut off values.

The median peak (range) concentration of C reactive protein was 62 mg/l (6–251) in the positive cultures overall and a median of 6 mg/l (6–139) in negative cultures. In the cultures that yielded *S epidermidis*, the peak C reactive protein was 44 (6–190) and in the other cultures a peak C reactive protein of 112 (44–251) was measured.

The effect on C reactive protein and band counts of events such as intraventricular haemorrhage, pneumothorax, intercostal drainage, and steroid administration was recorded. Of the five patients who had an intercostal drain insertion for pneumothorax, none had a recordable change in C reactive protein concentration. Sixteen patients had an intraventricular haemorrhage varying in severity from subependymal haemorrhage to intraventricular haemorrhage with associated parenchymal echodensity while daily C reactive protein was being measured. Only one

Table 4 Performance characteristics of acute phase indices at diagnostic cut off values: C reactive protein > 8mg/l, band >5%, and I:T ratio >0·2

Positive		*% Sensitivity*		*% Specificity*		*% Efficiency*		*Predictive accuracy (%)*
≤7 days	C reactive protein	55	(6/11)	88	(44/50)	82	(50/61)	50
	Bands	64	(7/11)	46*	(23/50)	49	(30/61)	21
	I:T ratio	55	(6/11)	24**	(35/50)	67	(41/61)	29
8–28 days	C reactive protein	68	(19/28)	72	(26/36)	70	(45/64)	66
	Bands	68	(19/28)	28*	(10/36)	45	(29/64)	41
	I:T ratio	33**	(9/27)	83	(30/36)	62	(39/63)	60
>28 days	C reactive protein	88	(15/17)	47	(14/30)	62	(29/47)	48
	Bands	76	(13/17)	40	(12/30)	53	(25/47)	42
	I:T ratio	24*	(4/17)	67	(20/30)	51	(24/47)	29
Overall	C reactive protein	71	(40/56)	72	(84/116)	72	(124/172)	56
	Bands	69	(39/56)	39*	(45/116)	49**	(84/172)	35**
	I:T ratio	34**	(19/55)	73	(85/116)	61	(104/171)	38
	Clinical	62	(35/56)	43*	(50/115)	50**	(85/171)	35**

Significant differences compared with C reactive protein in each postnatal age division: *=p<0,001, **p<0·05.

patient had a small increase in C reactive protein (11·2 mg/l) and another four had an increase but had concomitant infection. Four patients received dexamethasone with no change recorded in three but in one an increased C reactive protein was present because of infection which was being treated. Necrotising enterocolitis (intestinal pneumotosis and or perforation) occurred in three patients with a significant rise in C reactive protein. Two of three patients with transient abdominal distention of undetermined cause showed an increase in C reactive protein to 20–25 mg/l. Four patients had laparotomy and bowel resection for gastroschisis (n=2) and necrotising enterocolitis (n=2) and all had rapid and large increases in C reactive protein after surgery for up to five days. Daily intraventricular vancomycin by needle ventricular tap for the successful treatment of *S epidermidis* ventriculitis was associated with a steady decline in C reactive protein.

Discussion

There is a wide range of reported sensitivity and specificity for I:T ratio and C reactive protein due to variations in methods, study design, or the definition of infection.[3 7 10 11 19] Factors which may account for the reported variability of I:T ratio performance include: the I:T ratio is operator dependent, neutrophils are affected by non-infective events,[4] and I:T ratio is less sensitive after the first week of life.[20] Several factors may influence the performance of C reactive protein. First the timing of the sample in relation to the infection screen. Second, the measurement method used: quantitative measures have recently replaced semiquantitative methods.[12] Third, the concentration of C reactive protein regarded as diagnostic of infection varies between studies. Wassunna *et al,* using a sensitive solid phase ligand-banding radiometric immunoassay for C reactive protein, have established a normal range for cord blood and for the first 48 hours of life in preterm babies.[12] Infection was associated with large changes in C reactive protein within a range below the detection limit of most commercially available assays.

We have found the performance of serial C reactive protein (sensitivity 71% and specificity 72%) to be within the range reported by others.[5 10] Although there was not significant difference in sensitivity between C reactive protein and bands the specificity was significantly better for C reactive protein because the false positive rate was half that of the band count. The I:T ratio had a sensitivity of 34·5% and specificity of 73% which contrasts with 90% and 78% found by Philip and Hewitt[5] but is similar to the results of Kite *et al* who found a sensitivity of 29·4% and specificity of 81·5%.[10] The large difference in the I:T ratio sensitivity reported by Philip and Hewitt and our result is probably due to the difference in timing of the tests. Philip and Hewitt examined babies with suspected infection during the first seven days only, whereas our study involved babies of all ages. I:T ratio is not as accurate after the first week and our results do show a trend from a sensitivity of 55% in the first week to 24% after 28 days (table 4).

Using ROC, more comprehensive comparisons between laboratory test performance are possible.[15] Although a complete C reactive protein curve could not be constructed because no cut off values below 6 mg/l were determined, individual point comparisons were significantly different. The performance of C reactive protein as an index of infection is therefore superior to band and I:T ratio for any given diagnostic cut off level. This technique can be used to compare the performance of other quantitative tests.

The lack of response of C reactive protein to pneumothorax and intraventricular haemorrhage is similar to the report of Wassunna *et al,* who found no increase in C reactive protein after arterial catheterisation, endotracheal intubation, germinal matrix haemorrhage, or intraventricular haemorrhage.[12] The difference in response between bands (and I:T ratio) and C reactive protein when babies received dexamethasone is of interest and likely to be of clinical relevance. The rapid rise in all forms of polymorphonuclear leucocytes makes bands and I:T ratio invalid for the detection of infection during steroid therapy. C reactive protein did not rise during steroid treatment but in our study it was not possible to determine changes in C reactive protein concentration in the range below our detection limit (6 mg/l).

Although the specificity of clinical assessment was only 43%, two episodes of *E coli* septicaemia, which were not detected by the acute phase indicators (C reactive protein failed in one and bands in the other), were suspected by the rapid onset of clinical signs. In both cases, C reactive protein and bands showed an increase when measured soon after clinical signs were evident. The doubling time of C reactive protein is 4–6 hours and a more sensitive C reactive protein assay may have given an earlier warning of infection by demonstrating a rise in C reactive protein before reaching the level of our current assay detection limit. (Using a more sensitive technique and analysing daily samples retrospectively, we have recently observed a rise in C reactive protein occurring below the previous detection limit of 6 mg/l for 24–48 hours before infection was suspected.)

In neonatal units C reactive protein is therefore an adjunct to good clinical assessment,[12] but the development of a rapid and sensitive detection method is needed to exploit the potential of C reactive protein as an early marker of infection. (Our current assay takes a total of 45 minutes of laboratory time for an average of 20 samples from the neonatal unit.) Appropriate gestation and postnatal age specific normal ranges could be used to evaluate changes in C reactive protein over time in patients where there is clinical uncertainty. Changes in serial C reactive protein concentrations in babies at high risk of infection can

provide early evidence of infection, or confirm other non-specific infection indicators such as mild metabolic acidosis. However, a decision not to start antibiotics when clinically indicated cannot be based solely on a negative infection index, no matter how reliable the test. The advantage of serial quantitative C reactive protein tests are their objectivity and ready availability and this contrasts with the subjectivity of immature neutrophil estimations.

We conclude that *serial* C reactive protein estimation is significantly better than I:T ratio and percentage band count in detecting infection in preterm neonates. We plan to improve the detection limit for C reactive protein to be able to detect earlier changes in preterm infants at high risk of infection and to allow construction of complete C reactive protein ROC curves permitting more comprehensive comparisons with other tests. Using the ROC curve constructed with our current data we have determined an improved overall diagnostic cut off concentration of 7 mg/l to achieve a sensitivity of 75% (95% confidence interval 61 to 85%), specificity of 71% (59 to 83%) and a positive predictive accuracy of 55%.

1 Xanthou M. Leucocyte picture in healthy full-term and premature babies during the neonatal period. *Arch Dis Child* 1970;**45**:242–9.
2 Xanthou M. Leucocyte blood picture in ill newborn babies. *Arch Dis Child* 1972;**47**:741–6.
3 Gregory J, Hey E. Blood neutrophil response to bacterial infection in the first month of life. *Arch Dis Child* 1972;**47**:747–53.
4 Manroe BL, Weinberg AG, Rosenfield CR, Browne R. The neonatal blood count in health and disease. 1. Reference values for neutrophilic cells. *J Pediatr* 1979;**95**:89–98.
5 Philip AGS, Hewitt JR. Early diagnosis of neonatal sepsis. *Pediatrics* 1980;**65**:1036–41.
6 Speer CH, Bruns A, Gahr M. Sequential determination of CRP, α_1-antitrypsin and haptoglobin in neonatal septicaemia. *Acta Paediatr Scand* 1983;**72**:679–83.
7 Sabel K-G, Wadsworth Ch. C-reactive protein (CRP) in early diagnosis of neonatal septicaemia. *Acta Paediatr Scand* 1979;**68**:825–31.
8 Philip AGS. Acute-phase proteins in neonatal infection [Editorial]. *J Pediatr* 1984;**105**:940–2.
9 Lloyd BW, Oto A. Normal values for mature and immature neutrophils in very preterm babies. *Arch Dis Child* 1982; **57**:233–5.
10 Kite P, Millar MR, Gorham P, Congdon P. Comparison of five tests used in diagnosis of neonatal bacteraemia. *Arch Dis Child* 1988;**63**:639–43.
11 Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. *J Pediatr* 1988;**112**:761–7.
12 Wassunna A, Whitelaw A, Gallomore R, Hawkins PN, Pepys MB. C-reactive protein and bacterial infection in preterm infants. *Eur J Pediatr* 1990;**149**:424–7.
13 Hindocha P, Campbell CA, Gould JDM, Wojciechowski A, Wood CBS. Serial study of C reactive protein in neonatal septicaemia. *Arch Dis Child* 1984;**59**:435–8.
14 Sann L, Bienvenu F, Bienvenu J, Bourgeois J, Bethenod M. Evolution of serum prealbumin, C-reactive protein, and orosomucoid in neonates with bacterial infection. *J Pediatr* 1984;**105**:977–81.
15 Beck R, Schultz E. The use of relative operating characteristic (ROC) curves in test performance evaluation. *Arch Pathol Lab Med* 1986;**110**:13–20.
16 Gill CW, Bush WS, Burleigh WM, Fischer CL. An evaluation of a C-reactive protein assay using a rate immunonephelometric procedure. *Am J Clin Pathol* 1981; **75**: 50–5.
17 Machin D, Campbell MJ. *Statistical tables for the design of clinical trials.* Oxford: Blackwell Scientific, 1987.
18 Galen RS, Gambino SR. *Beyond normality: the predictive value and efficiency of medical diagnoses.* New York: John Wiley, 1975.
19 Siegel JD, McCracken GH. Sepsis neonatorum. *N Engl J Med* 1981;**304**:642–7.
20 Philip AGS. Detection of neonatal sepsis of late onset. *JAMA* 1982;**247**:489–92.