



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Goldfinch, CD;Korman, T;Kotsanas, D;Burgner, DP;Tan, K

Title:

C-reactive protein and immature-to-total neutrophil ratio have no utility in guiding lumbar puncture in suspected neonatal sepsis

Date:

2018-08-01

Citation:

Goldfinch, C. D., Korman, T., Kotsanas, D., Burgner, D. P. & Tan, K. (2018). C-reactive protein and immature-to-total neutrophil ratio have no utility in guiding lumbar puncture in suspected neonatal sepsis. *Journal of Paediatrics and Child Health*, 54 (8), pp.848-854. <https://doi.org/10.1111/jpc.13890>.

Persistent Link:

<https://hdl.handle.net/11343/283812>

# Inflammatory markers, the immature-to-total neutrophil ratio and the decision to perform a lumbar puncture in suspected neonatal sepsis

## ABSTRACT

**Introduction** Meningitis may complicate neonatal sepsis, but there is scant evidence to inform the decision to perform a lumbar puncture (LP) and considerable variation in practice. We investigated whether inflammatory markers – C-reactive protein (CRP) and immature-to-total neutrophil ratio (ITR) – were predictive of meningitis or significant CSF pleocytosis, and useful in guiding the decision to perform a LP.

**Methods** We studied all inpatients in a single tertiary neonatal unit < 6 months of age who had a LP performed between March 2011 and October 2014. We categorised CSF results as: (i) culture-positive meningitis, (ii) probable culture-negative meningitis but meeting *a priori* criteria for significant CSF leucocytosis, or (iii) no evidence of meningitis. CRP and ITR obtained within 48 hours of LP were analysed. We assessed the test performance of CRP and ITR by area under receiver operating characteristic curves (ROC).

**Results** 757 (male 471, 62.2%) infants were included. The median (IQR) gestational age was 38.4 weeks (30-40.3) and birth weight was 2940 (1330-3560) grams. Ten (1.3%) infants had culture-positive meningitis, 71 (9.4%) were classified as probable culture-negative meningitis, and 676 (89.3%) as non-meningitis. The area under ROC curve for culture-positive and probable culture-negative meningitis were 0.43 for CRP (95% CI 0.36-0.51) and 0.58 for ITR (0.51-0.65). At a CRP threshold of 30 mg/L, there was a positive likelihood ratio (LR) of 0.77, and a negative LR of 1.44.

**Conclusion** CRP and ITR perform poorly in identifying infants with confirmed or probable meningitis. The decision to perform a LP should be more focused on clinical grounds and/or a positive blood culture and less on inflammatory or haematological markers in isolation.

## Keywords

Meningitis, lumbar puncture, inflammatory markers, immature-to-total neutrophil ratio, C-reactive protein.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/jpc.13890](https://doi.org/10.1111/jpc.13890)



## INTRODUCTION

Neonatal meningitis has a significant long-term morbidity and mortality. Outcomes are worse if treatment is delayed.<sup>1,2</sup> The diagnosis is made by analysis of cerebrospinal fluid (CSF) obtained by lumbar puncture (LP), as clinical signs of meningitis observed in adults and older children are usually absent in infants. The clinical utility of LP in an infant should be balanced against potential adverse effects, such as the potential for clinical deterioration, pain and distress, false positive or equivocal results, and significant resource utilisation. Occasional complications of a LP include spinal haematoma or abscess, and cerebral herniation.<sup>3,4</sup> There is considerable variation in the decision to perform an LP in the evaluation of infants with early-onset<sup>5</sup> and late-onset<sup>5,6</sup> sepsis.

The American Academy of Pediatrics (AAP) guidelines recommend performing a LP in infants with a positive blood culture, and/or a clinical course or laboratory data (including raised inflammatory markers, for which no threshold is given) that strongly suggest bacterial sepsis, or in infants who deteriorate despite antimicrobial therapy.<sup>7</sup> Similarly, the UK National Institute for Clinical Excellence (NICE) guideline indicates that a LP should be considered in the context of clinical sepsis/meningitis, positive blood culture, no clinical improvement (despite treatment), and if the C-reactive protein (CRP) is > 10 mg/L.<sup>8</sup> There is an increased incidence of meningitis in bacteraemic infants, but performing an LP after blood cultures are positive may result in diagnostic delay or false negative CSF culture due to empiric antibiotic therapy.<sup>9,10</sup>

In our unit, a large metropolitan level 3 Neonatal Intensive Care Unit (NICU), we have adopted the AAP approach, generally performing a LP if there is a positive blood culture (except for coagulase negative staphylococci), if there is a clinical suspicion of meningitis, or if CRP is > 25-30 mg/L. In addition to CRP, we routinely measure the immature-to-total neutrophil ratio (ITR) and this may also influence some clinicians' decision to perform an LP. There is currently no evidence as to whether a threshold of CRP or ITR is informative for the diagnosis of meningitis in this context and if so, the cut-off at which LP should be performed.

**Aims**

Our primary aim was to investigate if elevated inflammatory markers (CRP and/or ITR) were predictive of meningitis in a population of inpatient newborn infants of all gestational ages (GA). The secondary aim was to determine if there was a clinically useful predictive cut-off at which to perform LP, in particular comparing the utility of our local CRP threshold of 30 mg/L with the NICE suggested threshold of >10 mg/L.

**METHODS****Study design**

This retrospective cohort study was conducted at Monash Newborn, Monash Children's Hospital (Victoria, Australia), a large tertiary level 3 NICU with 28 intensive care and 28 special care beds. The project was approved by the Quality Unit of the Monash Health Human Research Ethics Committee (#14374Q).

**Population**

The study population consisted of all infants admitted to the NICU or Special Care Nursery (SCN) at Monash Newborn < 6 months of age who had CSF samples sent to the Monash Health Pathology department between March 2011 and October 2014. If an infant had multiple CSF samples, only the first CSF sample was included in the analysis.

**Exclusion Criteria**

Exclusion criteria were age > 6 months at time of LP, CSF samples > 3mL volume (defined *a priori* to represent a shunt or sampling for treatment of hydrocephalus, rather than for diagnosis of possible meningitis). We anticipated that the remaining infants had a LP performed exclusively for exclusion of meningitis.

**Definitions**

### *Definitions of meningitis*

A large proportion of meningitis cases are diagnosed in the presence of negative CSF culture, as the LP is often performed after commencing antibiotics. We therefore derived case definitions based on secondary criteria of elevated CSF leucocytosis and developed an *a priori* probabilistic definition of neonatal meningitis, with infants categorised as follows (Figure 1):

1. *Confirmed culture-positive meningitis*, were defined by a CSF culture result that identified a bacteria that is not a contaminant. A coagulase negative staphylococcus on CSF culture was considered a contaminant in the absence of blood or second CSF culture of the same bacteria.
2. *Probable culture-negative meningitis*, were divided into four categories based on CSF white cell counts (WCC) and red cell counts (RCC):
  - a. Atraumatic (CSF RCC < 500/mm<sup>3</sup>), CSF WCC ≤ 100/mm<sup>3</sup>
  - b. Atraumatic (CSF RCC < 500/mm<sup>3</sup>), CSF WCC 20-99/mm<sup>3</sup>
  - c. Traumatic (CSF RCC ≤ 500/mm<sup>3</sup>), observed to predicted (O:P) CSF WCC:CSF RCC ratio of ≤ 10; based on the method of Greenberg *et al.*,<sup>11</sup>
$$O:P \text{ ratio} = \frac{CSF \text{ WCC observed} \times Peripheral \text{ RCC}}{CSF \text{ RCC} \times Peripheral \text{ WCC}}$$
  - d. Traumatic (CSF RCC ≤ 500/mm<sup>3</sup>), CSF WCC ≤ 100/mm<sup>3</sup>. This was based on our experience that traumatic samples with significantly elevated white cell counts are often treated as meningitis in clinical practice.
3. *Non-meningitis*; infants were considered not to have meningitis if they did not fit into any of the categories above, namely
  - a. CSF RCC < 500/mm<sup>3</sup>, CSF WCC < 20/mm<sup>3</sup>

- b. Traumatic samples with an O:P CSF WCC ratio of  $< 10$  and CSF WCC  $< 100/\text{mm}^3$

As there is no consensus for probable culture-negative meningitis, the threshold was based on data suggesting that a CSF WCC of  $> 100/\text{mm}^3$  had a sensitivity of 66% and a specificity of 94% (95% CI 93-95) for culture-positive meningitis.<sup>9</sup> We also included a definition with a lower WCC cut-off level of  $20/\text{mm}^3$ , as  $19/\text{mm}^3$  was the 95<sup>th</sup> percentile in a study of 142 infants aged 0-28 days who had CSF obtained in the emergency department but who did not have an underlying reason for a CSF pleocytosis, such as meningitis.<sup>12</sup>

We did not alter meningitis categories based on the results of viral studies as we felt that this was not reflective of the clinical reality, in that viral study results are typically unavailable when a decision to perform a LP and commence antibiotics is made.

### **Data collection**

Interrogation of the pathology database was performed for all infants who had CSF analysis as an inpatient between March 2011 and October 2014 on our NICU and SCN. We obtained data on CSF cell counts, viral studies (if available), Gram stain, and bacterial culture. The highest CRP and ITR value within 48 hours of CSF sampling, and blood culture results within 7 days of the CSF sample were obtained from the pathology database.

### **Data analysis**

We grouped infants as having meningitis if they fell into groups 1 and 2, based on the criteria described (Figure 1). We generated ROC curves, sensitivity and specificity analysis, and likelihood ratios at various thresholds and combinations of CRP and ITR. We also utilised median and interquartile range (IQR) to describe our data. Analysis was performed in STATA 12 (StataCorp, College Station TX).

## **RESULTS**

1193 CSF samples from 801 unique infants were identified from the pathology database. Samples were excluded if the infant previously had CSF sampling (n=392), if the infant was

aged > 6 months (n=4), if CSF volume was > 3mL (n=4), or if missing data made it impossible to categorise the outcome (n=36), leaving 757 unique samples (Figure 2). The median GA of included infants was 38.4 weeks (IQR 30-40.3), median BW was 2940 g (IQR 1330-3560g), and there were 471 males (62.2%). The median age at LP was day 2 of life (IQR 1-5); 475 (62.8%) LPs were performed in infants < 3 days of age, 236 (31.2%) 3 - 30 days, and 46 (6.1%) 30 days - 6 months of age. There were 341 (45.1%) traumatic samples (RCC median for traumatic samples 5850; IQR: 1620-33,500) (Table 1).

### **Culture-positive meningitis**

There were 11 infants with a positive CSF culture, one of which was subsequently considered a contaminant, leaving 10 infants (1.3%) identified as culture positive meningitis, all of whom were < 32 weeks gestational age and > 3 days of age. The median CSF WCC was 12 (IQR 6-202). CSF from 5 infants cultured coagulase negative staphylococci (CONS), including 4 with a concordant blood culture, and one infant in whom the blood culture was sterile and who was therefore categorised as not having meningitis. Of the infants who had CONS cultured in both their blood and CSF, all were significantly preterm (GA 24.1, 26, 26.3, 27.1) and treated as meningitis by their clinical team. None had central nervous system invasive procedures performed prior to their lumbar puncture and 1 infant had a subsequent lumbar puncture after treatment was commenced which showed no growth on blood culture. The median CSF WCC of the infants who grew CONS with a concordant blood culture was 2 (IQR 0-32). Two infants had CSF that cultured *Escherichia coli*, two had *Candida albicans* and one patient each had *Enterococcus faecalis* and *Enterobacter cloacae*. One infant with culture-positive meningitis had missing CRP and ITR data and was therefore excluded.

### **Blood cultures**

There were 117 (of 757, 15.5%) blood cultures sampled within 7 days of the LP which were positive for bacteria or fungi. Twenty-seven blood cultures yielded gram-negative bacteria, none of these infants had the same organism isolated on CSF culture. Eighty-five blood cultures yielded gram-positive bacteria, of which 54 were coagulase negative staphylococci. Of these, four infants had the same coagulase negative staphylococcus (on antibiogram)

isolated from their blood and CSF culture. Five blood cultures yielded a fungus, of which one infant had a positive CSF culture for the same fungus.

### **Probable (culture-negative) meningitis**

We identified 71 infants (9.3%) whose CSF met criteria for probable culture-negative meningitis. This included 13 infants with an atraumatic pleocytosis, of whom one had a CSF WCC  $\leq 100/\text{mm}^3$ , and 12 with a CSF WCC of 20-99/ $\text{mm}^3$ . The remaining 58 infants had traumatic CSF sampling (CSF RCC  $\leq 500/\text{mm}^3$ ), including 7 infants categorised on the basis of an O:P ratio  $\leq 10$ , and 51 infants who had a CSF WCC  $\leq 100/\text{mm}^3$ . There were 676 infants whose CSF samples were not indicative of meningitis.

### **Viral studies**

CSF from 55 infants had multiplex PCR for herpes viruses. Of these, cytomegalovirus was detected in one infant who had no leucocytes in the CSF and was classified as non-meningitis. CSF from 74 infants was tested for enterovirus by PCR, of which 3 were positive. Two of these infants had CSF WCC of 6 and 18, and were classified as non-meningitis. One infant had a CSF WCC of 62, and was classified as culture negative probable meningitis to reflect the clinical scenario at the time of decision to lumbar puncture.

### **Inflammatory markers**

We used area under ROC curves to analyse the utility of inflammatory markers in diagnosing confirmed or probable meningitis. Comparison using ROC curves of combined culture-positive and probable culture-negative meningitis categories with infants whose CSF was not suggestive of meningitis showed minimal evidence of the ability of CRP to discriminate combined culture positive and probable culture negative meningitis: CRP area under the curve (AUC) 0.43 (95% CI 0.36-0.51). Immature-to-total neutrophil ratio showed limited evidence of an ability to discriminate combined culture-positive and culture-negative meningitis: ITR AUC 0.58 (0.51-0.65). At a CRP threshold of 30 mg/L, the positive likelihood ratio was 0.77, and the negative likelihood ratio was 1.44. At an ITR threshold of

0.2, the positive likelihood ratio was 1.19, and negative likelihood ratio was 0.68. Likelihood ratios for varying thresholds for CRP and ITR are shown in Figure 3.

The test performance of CRP or ITR did not change with the different definitions of meningitis: culture-positive meningitis versus all culture-negative CRP AUC 0.56 (95% CI 0.49-0.64), ITR AUC 0.62 (0.55-0.69); culture-positive meningitis and culture-negative CSF WCC  $\geq 100$  versus all other infants CRP AUC 0.52 (0.33-0.72), ITR AUC 0.57 (0.40-0.74) (Table 2).

We performed a sub-group analysis by dividing our population by GA into  $\geq 30$  and  $< 30$  week categories, but this did not improve the test performance of CRP or ITR in identifying combined culture positive and culture negative meningitis: GA  $\geq 30$  CRP AUC 0.52 (95% CI 0.41-0.63), ITR AUC 0.65 (0.59-0.72); GA  $< 30$  CRP AUC 0.43 (0.33-0.54), ITR AUC 0.54 (0.42-0.66).

We also assessed the test performance of using any abnormal inflammatory marker (CRP  $\geq 30$  or ITR  $\geq 0.2$ ) but this showed poor ability to discriminate combined culture-positive and culture-negative meningitis: positive likelihood ratio 1.35, negative likelihood ratio 0.97.

## DISCUSSION

This is the first study to investigate the value of CRP and ITR in informing the decision to perform a LP in preterm and term infants. Both parameters had poor diagnostic test performance for the prediction of culture proven or probable meningitis. We found that CRP was not associated with culture proven or probable culture negative meningitis, and ITR was only modestly associated. Neither parameter had clinically useful performance characteristics in our population. Our findings suggest that neither CRP nor ITR should guide the decision to perform a LP in infants with suspected neonatal sepsis.

### Strengths and limitations

The strengths of the study include analysis a large population of inpatients in a tertiary neonatal unit over 3.5 years. The unit serves a multicultural population in south-eastern Melbourne and receives outborn infants from other smaller centres. We defined criteria for probable culture-negative meningitis *a priori*, based on data from Garges<sup>9</sup> and Kestenbaum<sup>12</sup>, and our experience that the majority of meningitis diagnoses are made on the basis of CSF pleocytosis (rather than positive bacterial culture) due to prior antibiotic administration. A range of *a priori* definitions for probable culture-negative meningitis were used; none improved the test performance of CRP or ITR.

We acknowledge some limitations of this retrospective single-centre study, including possible differences in defining and managing neonatal infection in other centres, which may limit generalisability. Analogous studies in other settings are warranted. We were unable to use culture-proven meningitis as the sole outcome measure, as this is now rare in most neonatal units, where empiric antibiotics are often commenced prior to a LP being performed;<sup>13, 14</sup> intravenous antibiotics rapidly sterilise the CSF.<sup>15</sup> We therefore used various definitions of CSF pleocytosis as a surrogate for meningitis, but the sensitivity and specificity of these definitions for actual meningitis in our population are impossible to establish. Almost half of infants had traumatic CSF samples; there is no consensus in the literature regarding interpretation of traumatic samples. We used published data<sup>11</sup> to define O:P ratios to categorise traumatic CSF samples. Most of CSF samples were obtained within 48 hours of

birth (to exclude early onset meningitis). We are aware of maturational effect of postnatal age on the normative values of WCC in the CSF of newborn infants<sup>16</sup>, however, as most LPs were performed shortly after birth, we lacked power to perform additional analysis by postnatal age. As we did not obtain patient-level clinical data, we could not determine the timing of the LP relative to commencing antibiotics. However in our unit and elsewhere, pleocytosis in a CSF sample obtained after antibiotics are commenced is often used to determine the need for a treatment course of antibiotics for presumed meningitis. Therefore the findings have real-world clinical applicability in informing criteria on which the decision to perform an LP are made.

Finally, the clinical practice in our unit during the study period was to perform an LP on clinical grounds, a positive blood culture, or if the CRP was >25-30 mg/L. We therefore cannot comment on the incidence of meningitis or CSF pleocytosis in infants not meeting these criteria; such a study would require obtaining CSF in infants in whom it was not thought to be clinically warranted, which is unethical. Further studies that investigate the predictors of meningitis and significant CSF pleocytosis in infants selected on blood culture positivity or clinical grounds alone (but not on CRP or ITR) may partially address this issue.

### **Relationship to prior literature**

Our study is the first to assess the relationship between CRP and ITR and neonatal meningitis.

### **Unanswered questions**

Further studies should address what clinical and possibly laboratory criteria are predictive of abnormal CSF if the decision to perform a LP is made on clinical grounds and/or a positive blood culture. A prospective study with CSF obtained prior to antibiotics would define meaningful thresholds for CSF pleocytosis and interpretation of traumatic LP. Such studies would be difficult to perform in practice as antibiotics are often commenced prior to the decision to perform an LP.

### **Conclusions**

Peripheral inflammatory markers, CRP and ITR, either individually or in combination, have poor test performance in diagnosing culture-positive and probable culture-negative meningitis. The decision to perform a LP should be more focused on clinical grounds and/or a positive blood culture and less on inflammatory or haematological markers in isolation.

## **ACKNOWLEDGEMENTS**

### **Financial disclosure statement**

The authors have no financial relationships relevant to this article to disclose

### **Funding**

Study authors are clinical employees of Monash Health. DPB is an NHMRC Senior Research Fellow and NHFA Honorary Future Leader Fellow. This study required no specific funding support.

### **Potential conflicts of interest**

The authors have no conflicts of interest relevant to this article to disclose

### **Competing interests**

None

## REFERENCES

- [1] Bortolussi R, Krishnan C, Armstrong D, Tovichayathamrong P. Prognosis for survival in neonatal meningitis: clinical and pathologic review of 52 cases. *Canadian Medical Association journal*. 1978; **118**: 165-168.
- [2] Klinger G, Chin CN, Beyene J, Perlman M. Predicting the outcome of neonatal bacterial meningitis. *Pediatrics*. 2000; **106**: 477-482.
- [3] Potgieter S, Dimin S, Lagae L, et al. Epidermoid tumours associated with lumbar punctures performed in early neonatal life. *Developmental medicine and child neurology*. 1998; **40**: 266-269.
- [4] Kalay S, Öztekin O, Tezel G, Demirtaş H, Akçakuş M, Oygür N. Cerebellar Herniation after Lumbar Puncture in Galactosemic Newborn. *AJP Reports*. 2011; **1**: 43-46.
- [5] Patrick SW, Schumacher RE, Davis MM. Variation in lumbar punctures for early onset neonatal sepsis: a nationally representative serial cross-sectional analysis, 2003-2009. *BMC pediatrics*. 2012; **12**: 134.
- [6] Joshi P, Barr P. The use of lumbar puncture and laboratory tests for sepsis by Australian neonatologists. *Journal of paediatrics and child health*. 1998; **34**: 74-78.
- [7] Polin RA, Committee on F, Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. *Pediatrics*. 2012; **129**: 1006-1015.
- [8] Caffrey Osvald E, Prentice P. NICE clinical guideline: antibiotics for the prevention and treatment of early-onset neonatal infection. *Archives of disease in childhood Education and practice edition*. 2014; **99**: 98-100.
- [9] Garges HP, Moody MA, Cotten CM, et al. Neonatal meningitis: what is the correlation among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters? *Pediatrics*. 2006; **117**: 1094-1100.
- [10] Stoll BJ, Hansen N, Fanaroff AA, et al. To tap or not to tap: high likelihood of meningitis without sepsis among very low birth weight infants. *Pediatrics*. 2004; **113**: 1181-1186.
- [11] Greenberg RG, Smith PB, Cotten CM, Moody MA, Clark RH, Benjamin DK, Jr. Traumatic lumbar punctures in neonates: test performance of the cerebrospinal fluid white blood cell count. *The Pediatric infectious disease journal*. 2008; **27**: 1047-1051.
- [12] Kestenbaum LA, Ebberson J, Zorc JJ, Hodinka RL, Shah SS. Defining cerebrospinal fluid white blood cell count reference values in neonates and young infants. *Pediatrics*. 2010; **125**: 257-264.
- [13] Nigrovic LE, Malley R, Macias CG, et al. Effect of antibiotic pretreatment on cerebrospinal fluid profiles of children with bacterial meningitis. *Pediatrics*. 2008; **122**: 726-730.
- [14] Geiseler PJ, Nelson KE, Levin S, Reddi KT, Moses VK. Community-acquired purulent meningitis: a review of 1,316 cases during the antibiotic era, 1954-1976. *Reviews of infectious diseases*. 1980; **2**: 725-745.
- [15] Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics*. 2001; **108**: 1169-1174.
- [16] Connell T, Curtis N. How to Interpret a CSF—The Art and the Science. In: Pollard AJ, Finn A, eds. *Hot Topics in Infection and Immunity in Children II*. Springer US, Boston, MA, 2005; 199-216.

	All infants	Culture proven meningitis †	Culture negative probable meningitis				Sub-total	Non-meningitis ‡
			Atraumatic CSF WCC e 100 /mm <sup>3</sup>	Atraumatic CSF WCC 20-99 /mm <sup>3</sup>	Traumatic OP ratio e 10	Traumatic CSF WCC e 100 /mm <sup>3</sup>		
Male, no (%)	471 (62)	7 (70)	1 (100)	6 (50)	2 (29)	35 (69)	44 (62)	420 (62)
Gestation median (IQR)	38.4 (30.0-40.3)	26.3 (24.3-28.1)	27.4	37.4 (29.9-39.9)	29.6 (28.9-39.6)	30.6 (28.0-38.6)	30.6 (28-39.6)	38.7 (30.6-40.4)
Birth weight(g) (IQR)	2940 (1330-3560)	880 (770-1300)	1110	2810 (1030-2890)	1170 (1160-3040)	1490 (1240-3380)	1490 (1130-3050)	3010 (1440-3600)
Age								
< 3 days no. (%)	475 (63)	0 (0)		4 (33)	5 (71)	40 (78)	49 (69)	426 (63)
3-30 days no. (%)	236 (31)	7 (70)	1 (100)	6 (50)	2 (29)	9 (18)	18 (23)	211 (31)
1-6 months no. (%)	46 (6)	3 (30)		2 (17)		2 (4)	4 (6)	39 (6)
CRP median (IQR)	37.7 (24.3-57.3)	33.7 (21.2-63.1)	74.1	37.4 (30.6-55.2)	4.2 (1.2-37.8)	28.2 (8.2-56.9)	30.6 (8.2-55.7)	38.2 (25.3-57.4)
ITR median (IQR)	0.28 (0.14-0.46)	0.39 (0.22-0.61)	0.60	0.31 (0.07-0.48)	0.33 (0.07-0.45)	0.37 (0.25-0.55)	0.34 (0.19-0.53)	0.27 (0.13-0.45)
pWCC median (IQR)	15.1 (9.9-20.6)	16.3 (8.8-20.3)	8.5	16.1 (13.7-19.9)	10.5 (8.3-13.4)	17.8 (14.1-25.8)	17.0 (12.4-20.9)	15.0 (9.8-20.5)
pNeut median (IQR)	5.8 (3.3-9.5)	5.8 (1.5-8.4)	2.4	5.7 (3.7-9.5)	4.0 (3.3-5.5)	6.7 (3.1-9.9)	5.7 (3.3-9.4)	5.9 (3.3-9.7)
Blood culture pos. no. (%)	117 (16)	6 (60)	1 (100)	4 (33)	1 (14)	6 (12)	12 (17)	99 (15)

Total samples (n)	757	10	1	12	7	51	71	676
----------------------	-----	----	---	----	---	----	----	-----

Table 1: Table of baseline characteristics for infants by meningitis category.

*CSF indicates cerebrospinal fluid; WCC white cell count; no. Number; pWCC peripheral white cell count, pNeut peripheral neutrophil count, IQR interquartile range. P values in culture proven meningitis column are comparing culture proven meningitis vs all culture-negative. P values in total probable culture-negative column are comparing culture-positive and culture-negative meningitis vs non-meningitis. FBE full blood examination*

† *1 infant did not have FBE or CRP measurements*

‡ *5 infants were missing both CRP and ITR, 2 infants were missing CRP only*

	Meningitis	Probable culture negative meningitis	Non-meningitis	
CRP < 30	4	35	226	265
CRP e 30	5	36	443	484
	9	71	669	749 †

	Meningitis	Probable culture negative meningitis	Non-meningitis	
ITR < 0.2	2	18	248	268
ITR e 0.2	7	53	423	483
Total	9	71	671	751 ‡

Table 2: Contingency 2x3 table for cut-offs of C-reactive protein 30 mg/L and for immature-to-total neutrophil ratio 0.2.

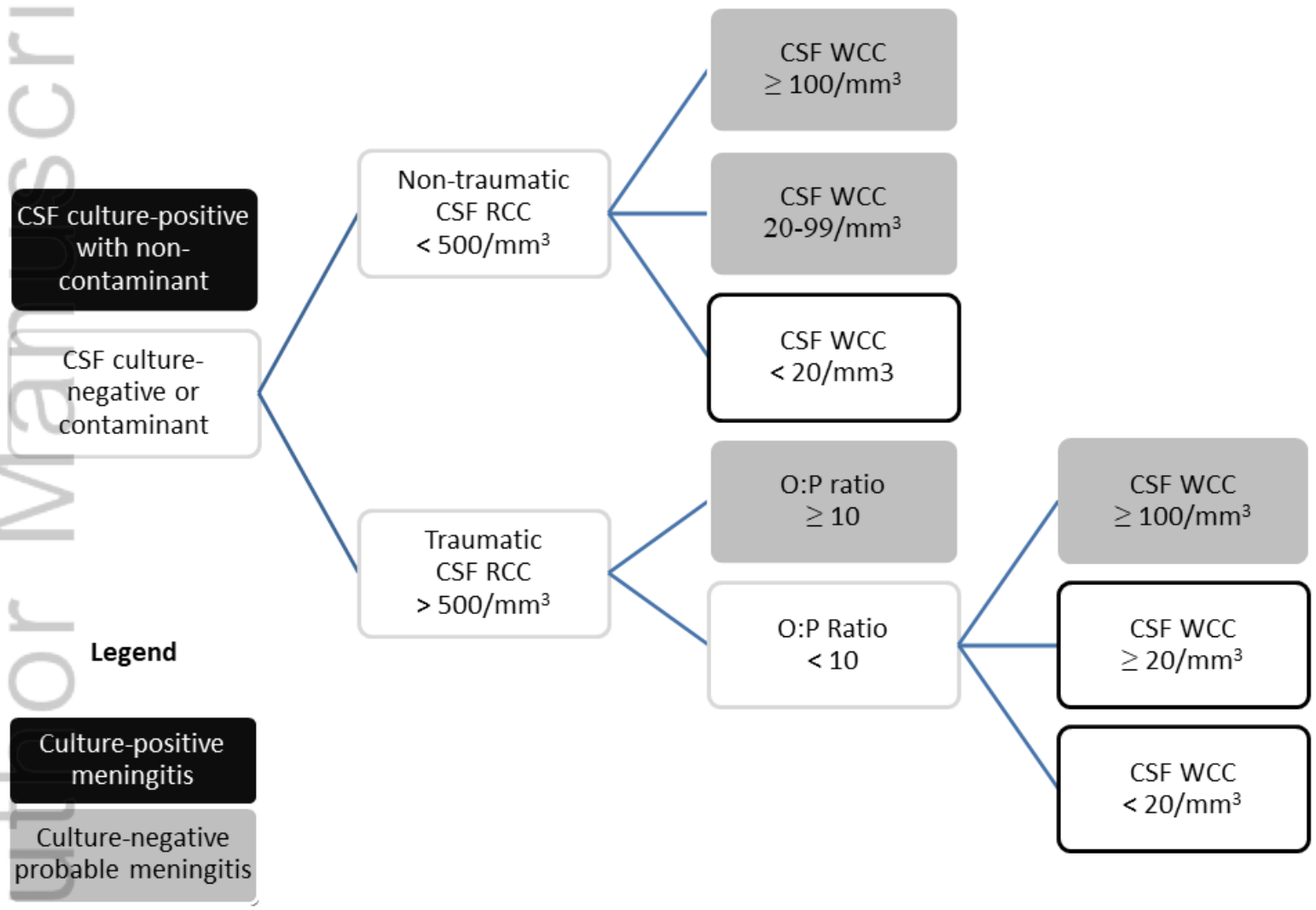
*CRP, C-reactive protein; ITR, immature-to-total neutrophil ratio † 8 infants did not have CRP measurements ‡ 6 infants did not have ITR measurements*

## FIGURE LEGENDS

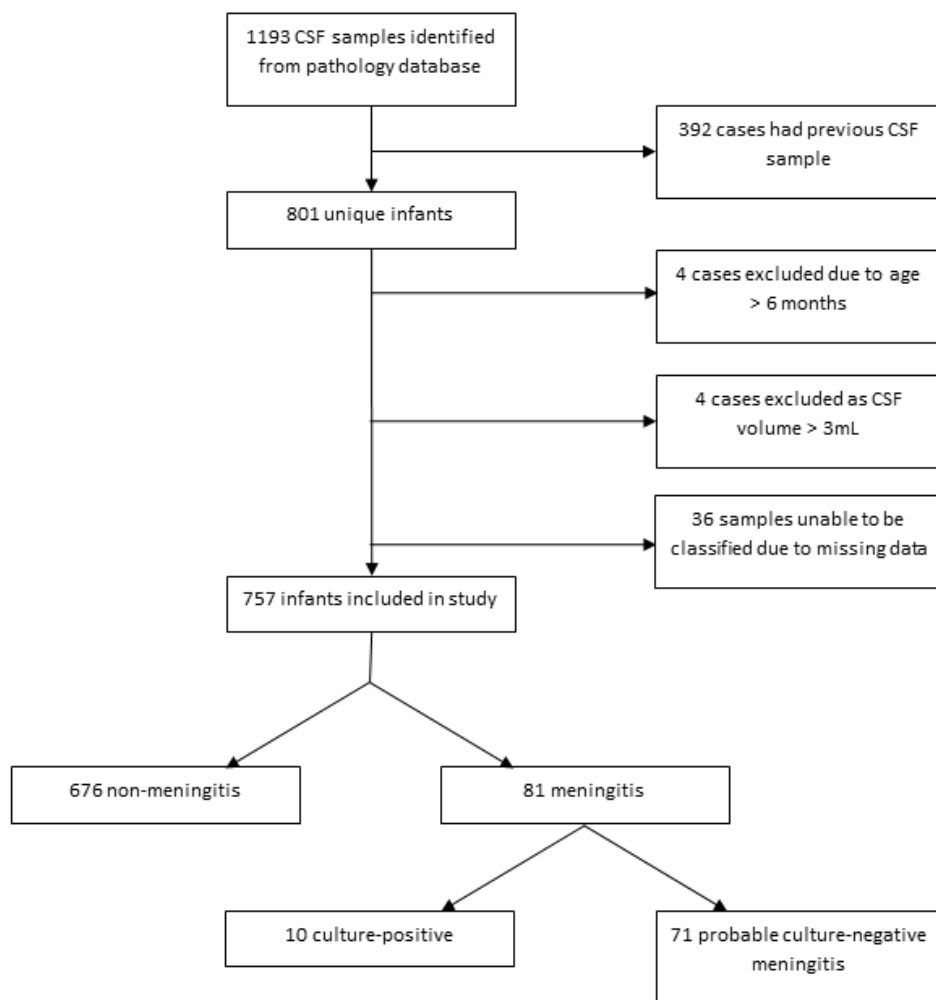
Figure 1: Flowchart for definitions of meningitis.

Figure 2. Study flow diagram

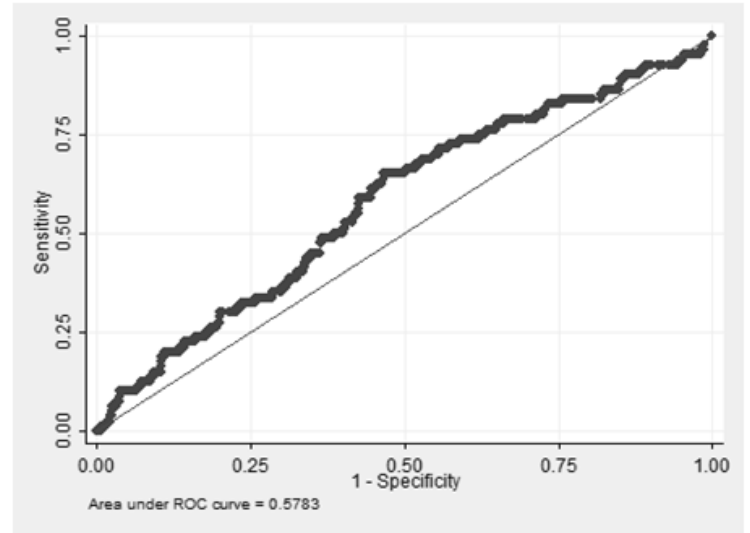
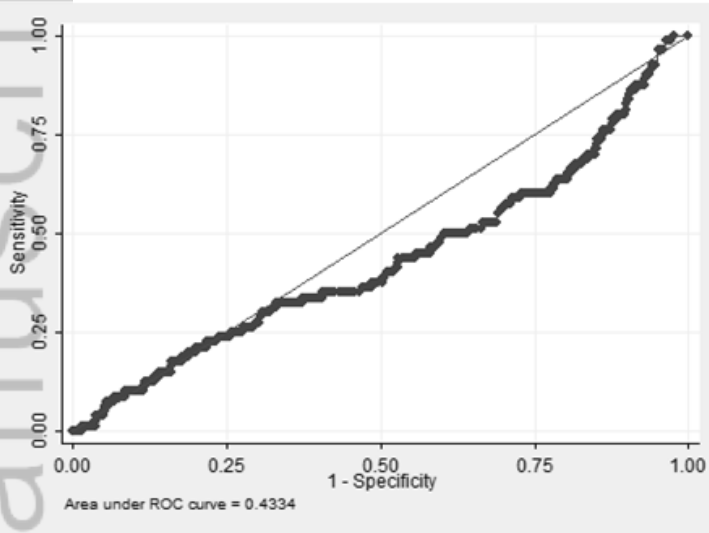
Figure 3: C-reactive protein and immature-to-total neutrophil ratio receiver operating characteristic curves and likelihood ratios. CRP indicates C-reactive protein; ITR immature-to-total neutrophil ratio, LR+ positive likelihood ratio, LR- negative likelihood ratio, CI confidence interval, AUC area under the receiver operating characteristic curve



Figures 1.tif



Figures 2.tif



	Threshold	LR+ (95% CI)	LR- (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
<b>CRP (mg/L)</b>	10	0.88 (0.78-1.00)	1.75 (1.13-2.70)	76.3 (65.4-85.1)	13.6 (11.1-16.4)
	30	0.77 (0.62-0.96)	1.44 (1.13-1.85)	51.2 (39.8-62.6)	33.8 (30.2-37.5)
	100	1.05 (0.52-2.10)	1.00 (0.92-1.07)	10.0 (4.4-18.8)	90.4 (87.9-92.6)
<b>ITR</b>	0.2	1.19 (1.04-1.37)	0.68 (0.46-1.00)	75.0 (64.1-84.0)	37.0 (33.3-40.7)
	0.3	1.37 (1.13-1.66)	0.69 (0.52-0.92)	62.5 (51.0-73.1)	54.4 (50.5-58.2)
	0.6	1.68 (1.03-2.72)	0.91 (0.81-1.02)	20.0 (11.9-30.4)	88.1 (85.4-90.4)

Figures 3.tif

# Inflammatory markers, the immature-to-total neutrophil ratio and the decision to perform a lumbar puncture in suspected neonatal sepsis

## ABSTRACT

**Introduction** Meningitis may complicate neonatal sepsis, but there is scant evidence to inform the decision to perform a lumbar puncture (LP) and considerable variation in practice. We investigated whether inflammatory markers – C-reactive protein (CRP) and immature-to-total neutrophil ratio (ITR) – were predictive of meningitis or significant CSF pleocytosis, and useful in guiding the decision to perform a LP.

**Methods** We studied all inpatients in a single tertiary neonatal unit < 6 months of age who had a LP performed between March 2011 and October 2014. We categorised CSF results as: (i) culture-positive meningitis, (ii) probable culture-negative meningitis but meeting *a priori* criteria for significant CSF leucocytosis, or (iii) no evidence of meningitis. CRP and ITR obtained within 48 hours of LP were analysed. We assessed the test performance of CRP and ITR by area under receiver operating characteristic curves (ROC).

**Results** 757 (male 471, 62.2%) infants were included. The median (IQR) gestational age was 38.4 weeks (30-40.3) and birth weight was 2940 (1330-3560) grams. Ten (1.3%) infants had culture-positive meningitis, 71 (9.4%) were classified as probable culture-negative meningitis, and 676 (89.3%) as non-meningitis. The area under ROC curve for culture-positive and probable culture-negative meningitis were 0.43 for CRP (95% CI 0.36-0.51) and 0.58 for ITR (0.51-0.65). At a CRP threshold of 30 mg/L, there was a positive likelihood ratio (LR) of 0.77, and a negative LR of 1.44.

**Conclusion** CRP and ITR perform poorly in identifying infants with confirmed or probable meningitis. The decision to perform a LP should be more focused on clinical grounds and/or a positive blood culture and less on inflammatory or haematological markers in isolation.

## Keywords

Meningitis, lumbar puncture, inflammatory markers, immature-to-total neutrophil ratio, C-reactive protein.

## INTRODUCTION

Neonatal meningitis has a significant long-term morbidity and mortality. Outcomes are worse if treatment is delayed.<sup>1,2</sup> The diagnosis is made by analysis of cerebrospinal fluid (CSF) obtained by lumbar puncture (LP), as clinical signs of meningitis observed in adults and older children are usually absent in infants. The clinical utility of LP in an infant should be balanced against potential adverse effects, such as the potential for clinical deterioration, pain and distress, false positive or equivocal results, and significant resource utilisation. Occasional complications of a LP include spinal haematoma or abscess, and cerebral herniation.<sup>3,4</sup> There is considerable variation in the decision to perform an LP in the evaluation of infants with early-onset<sup>5</sup> and late-onset<sup>5,6</sup> sepsis.

The American Academy of Pediatrics (AAP) guidelines recommend performing a LP in infants with a positive blood culture, and/or a clinical course or laboratory data (including raised inflammatory markers, for which no threshold is given) that strongly suggest bacterial sepsis, or in infants who deteriorate despite antimicrobial therapy.<sup>7</sup> Similarly, the UK National Institute for Clinical Excellence (NICE) guideline indicates that a LP should be considered in the context of clinical sepsis/meningitis, positive blood culture, no clinical improvement (despite treatment), and if the C-reactive protein (CRP) is  $> 10$  mg/L.<sup>8</sup> There is an increased incidence of meningitis in bacteraemic infants, but performing an LP after blood cultures are positive may result in diagnostic delay or false negative CSF culture due to empiric antibiotic therapy.<sup>9,10</sup>

In our unit, a large metropolitan level 3 Neonatal Intensive Care Unit (NICU), we have adopted the AAP approach, generally performing a LP if there is a positive blood culture (except for coagulase negative staphylococci), if there is a clinical suspicion of meningitis, or if CRP is  $> 25$ - $30$  mg/L. In addition to CRP, we routinely measure the immature-to-total neutrophil ratio (ITR) and this may also influence some clinicians' decision to perform an LP. There is currently no evidence as to whether a threshold of CRP or ITR is informative for the diagnosis of meningitis in this context and if so, the cut-off at which LP should be performed.

### Aims

Our primary aim was to investigate if elevated inflammatory markers (CRP and/or ITR) were

predictive of meningitis in a population of inpatient newborn infants of all gestational ages (GA). The secondary aim was to determine if there was a clinically useful predictive cut-off at which to perform LP, in particular comparing the utility of our local CRP threshold of 30 mg/L with the NICE suggested threshold of >10 mg/L.

## **METHODS**

### **Study design**

This retrospective cohort study was conducted at Monash Newborn, Monash Children's Hospital (Victoria, Australia), a large tertiary level 3 NICU with 28 intensive care and 28 special care beds. The project was approved by the Quality Unit of the Monash Health Human Research Ethics Committee (#14374Q).

### **Population**

The study population consisted of all infants admitted to the NICU or Special Care Nursery (SCN) at Monash Newborn < 6 months of age who had CSF samples sent to the Monash Health Pathology department between March 2011 and October 2014. If an infant had multiple CSF samples, only the first CSF sample was included in the analysis.

### **Exclusion Criteria**

Exclusion criteria were age > 6 months at time of LP, CSF samples > 3mL volume (defined *a priori* to represent a shunt or sampling for treatment of hydrocephalus, rather than for diagnosis of possible meningitis). We anticipated that the remaining infants had a LP performed exclusively for exclusion of meningitis.

### **Definitions**

#### *Definitions of meningitis*

A large proportion of meningitis cases are diagnosed in the presence of negative CSF culture, as the LP is often performed after commencing antibiotics. We therefore derived case definitions based on secondary criteria of elevated CSF leucocytosis and developed an *a priori* probabilistic definition of neonatal meningitis, with infants categorised as follows (Figure 1):

1. *Confirmed culture-positive meningitis*, were defined by a CSF culture result that identified a bacteria that is not a contaminant. A coagulase negative staphylococcus on CSF culture was considered a contaminant in the absence of blood or second CSF culture of the same bacteria.
2. *Probable culture-negative meningitis*, were divided into four categories based on CSF white cell counts (WCC) and red cell counts (RCC):
  - a. Atraumatic (CSF RCC < 500/mm<sup>3</sup>), CSF WCC  $\leq$  100/mm<sup>3</sup>
  - b. Atraumatic (CSF RCC < 500/mm<sup>3</sup>), CSF WCC 20-99/mm<sup>3</sup>
  - c. Traumatic (CSF RCC  $\leq$  500/mm<sup>3</sup>), observed to predicted (O:P) CSF WCC:CSF RCC ratio of  $\leq$  10; based on the method of Greenberg *et al.*,<sup>11</sup>

$$O:P \text{ ratio} = \frac{CSF \text{ WCC observed} \times Peripheral \text{ RCC}}{CSF \text{ RCC} \times Peripheral \text{ WCC}}$$
  - d. Traumatic (CSF RCC  $\leq$  500/mm<sup>3</sup>), CSF WCC  $\leq$  100/mm<sup>3</sup>. This was based on our experience that traumatic samples with significantly elevated white cell counts are often treated as meningitis in clinical practice.
3. *Non-meningitis*; infants were considered not to have meningitis if they did not fit into any of the categories above, namely
  - a. CSF RCC < 500/mm<sup>3</sup>, CSF WCC < 20/mm<sup>3</sup>
  - b. Traumatic samples with an O:P CSF WCC ratio of < 10 and CSF WCC < 100/mm<sup>3</sup>

As there is no consensus for probable culture-negative meningitis, the threshold was based on data suggesting that a CSF WCC of > 100/mm<sup>3</sup> had a sensitivity of 66% and a specificity of 94% (95% CI 93-95) for culture-positive meningitis.<sup>9</sup> We also included a definition with a lower WCC cut-off level of 20/mm<sup>3</sup>, as 19/mm<sup>3</sup> was the 95<sup>th</sup> percentile in a study of 142 infants aged 0-28 days who had CSF obtained in the emergency department but who did not have an underlying reason for a CSF pleocytosis, such as meningitis.<sup>12</sup>

We did not alter meningitis categories based on the results of viral studies as we felt that this was not reflective of the clinical reality, in that viral study results are typically unavailable when a decision to perform a LP and commence antibiotics is made.

## **Data collection**

Interrogation of the pathology database was performed for all infants who had CSF analysis as an inpatient between March 2011 and October 2014 on our NICU and SCN. We obtained data on CSF cell counts, viral studies (if available), Gram stain, and bacterial culture. The highest CRP and ITR value within 48 hours of CSF sampling, and blood culture results within 7 days of the CSF sample were obtained from the pathology database.

## **Data analysis**

We grouped infants as having meningitis if they fell into groups 1 and 2, based on the criteria described (Figure 1). We generated ROC curves, sensitivity and specificity analysis, and likelihood ratios at various thresholds and combinations of CRP and ITR. We also utilised median and interquartile range (IQR) to describe our data. Analysis was performed in STATA 12 (StataCorp, College Station TX).

## **RESULTS**

1193 CSF samples from 801 unique infants were identified from the pathology database. Samples were excluded if the infant previously had CSF sampling (n=392), if the infant was aged > 6 months (n=4), if CSF volume was > 3mL (n=4), or if missing data made it impossible to categorise the outcome (n=36), leaving 757 unique samples (Figure 2). The median GA of included infants was 38.4 weeks (IQR 30-40.3), median BW was 2940 g (IQR 1330-3560g), and there were 471 males (62.2%). The median age at LP was day 2 of life (IQR 1-5); 475 (62.8%) LPs were performed in infants < 3 days of age, 236 (31.2%) 3 - 30 days, and 46 (6.1%) 30 days - 6 months of age. There were 341 (45.1%) traumatic samples (RCC median for traumatic samples 5850; IQR: 1620-33,500) (Table 1).

### **Culture-positive meningitis**

There were 11 infants with a positive CSF culture, one of which was subsequently considered a contaminant, leaving 10 infants (1.3%) identified as culture positive meningitis, all of whom were < 32 weeks gestational age and > 3 days of age. The median CSF WCC was 12 (IQR 6-202). CSF from 5 infants cultured coagulase negative staphylococci (CONS), including 4 with a concordant blood culture, and one infant in whom the blood culture was sterile and who was therefore categorised as not having meningitis. Of the infants who had CONS cultured in both their blood and CSF, all were significantly preterm (GA 24.1, 26,

26.3, 27.1) and treated as meningitis by their clinical team. None had central nervous system invasive procedures performed prior to their lumbar puncture and 1 infant had a subsequent lumbar puncture after treatment was commenced which showed no growth on blood culture. The median CSF WCC of the infants who grew CONS with a concordant blood culture was 2 (IQR 0-32). Two infants had CSF that cultured *Escherichia coli*, two had *Candida albicans* and one patient each had *Enterococcus faecalis* and *Enterobacter cloacae*. One infant with culture-positive meningitis had missing CRP and ITR data and was therefore excluded.

### **Blood cultures**

There were 117 (of 757, 15.5%) blood cultures sampled within 7 days of the LP which were positive for bacteria or fungi. Twenty-seven blood cultures yielded gram-negative bacteria, none of these infants had the same organism isolated on CSF culture. Eighty-five blood cultures yielded gram-positive bacteria, of which 54 were coagulase negative staphylococci. Of these, four infants had the same coagulase negative staphylococcus (on antibiogram) isolated from their blood and CSF culture. Five blood cultures yielded a fungus, of which one infant had a positive CSF culture for the same fungus.

### **Probable (culture-negative) meningitis**

We identified 71 infants (9.3%) whose CSF met criteria for probable culture-negative meningitis. This included 13 infants with an atraumatic pleocytosis, of whom one had a CSF WCC  $\leq 100/\text{mm}^3$ , and 12 with a CSF WCC of 20-99/ $\text{mm}^3$ . The remaining 58 infants had traumatic CSF sampling (CSF RCC  $\leq 500/\text{mm}^3$ ), including 7 infants categorised on the basis of an O:P ratio  $\leq 10$ , and 51 infants who had a CSF WCC  $\leq 100/\text{mm}^3$ . There were 676 infants whose CSF samples were not indicative of meningitis.

### **Viral studies**

CSF from 55 infants had multiplex PCR for herpes viruses. Of these, cytomegalovirus was detected in one infant who had no leucocytes in the CSF and was classified as non-meningitis. CSF from 74 infants was tested for enterovirus by PCR, of which 3 were positive. Two of these infants had CSF WCC of 6 and 18, and were classified as non-meningitis. One infant had a CSF WCC of 62, and was classified as culture negative probable meningitis to reflect the clinical scenario at the time of decision to lumbar puncture.

### **Inflammatory markers**

We used area under ROC curves to analyse the utility of inflammatory markers in diagnosing confirmed or probable meningitis. Comparison using ROC curves of combined culture-positive and probable culture-negative meningitis categories with infants whose CSF was not suggestive of meningitis showed minimal evidence of the ability of CRP to discriminate combined culture positive and probable culture negative meningitis: CRP area under the curve (AUC) 0.43 (95% CI 0.36-0.51). Immature-to-total neutrophil ratio showed limited evidence of an ability to discriminate combined culture-positive and culture-negative meningitis: ITR AUC 0.58 (0.51-0.65). At a CRP threshold of 30 mg/L, the positive likelihood ratio was 0.77, and the negative likelihood ratio was 1.44. At an ITR threshold of 0.2, the positive likelihood ratio was 1.19, and negative likelihood ratio was 0.68. Likelihood ratios for varying thresholds for CRP and ITR are shown in Figure 3.

The test performance of CRP or ITR did not change with the different definitions of meningitis: culture-positive meningitis versus all culture-negative CRP AUC 0.56 (95% CI 0.49-0.64), ITR AUC 0.62 (0.55-0.69); culture-positive meningitis and culture-negative CSF WCC  $\geq 100$  versus all other infants CRP AUC 0.52 (0.33-0.72), ITR AUC 0.57 (0.40-0.74) (Table 2).

We performed a sub-group analysis by dividing our population by GA into  $\geq 30$  and  $< 30$  week categories, but this did not improve the test performance of CRP or ITR in identifying combined culture positive and culture negative meningitis: GA  $\geq 30$  CRP AUC 0.52 (95% CI 0.41-0.63), ITR AUC 0.65 (0.59-0.72); GA  $< 30$  CRP AUC 0.43 (0.33-0.54), ITR AUC 0.54 (0.42-0.66).

We also assessed the test performance of using any abnormal inflammatory marker (CRP  $\geq 30$  or ITR  $\geq 0.2$ ) but this showed poor ability to discriminate combined culture-positive and culture-negative meningitis: positive likelihood ratio 1.35, negative likelihood ratio 0.97.

## DISCUSSION

This is the first study to investigate the value of CRP and ITR in informing the decision to perform a LP in preterm and term infants. Both parameters had poor diagnostic test performance for the prediction of culture proven or probable meningitis. We found that CRP was not associated with culture proven or probable culture negative meningitis, and ITR was only modestly associated. Neither parameter had clinically useful performance characteristics in our population. Our findings suggest that neither CRP nor ITR should guide the decision to perform a LP in infants with suspected neonatal sepsis.

### Strengths and limitations

The strengths of the study include analysis a large population of inpatients in a tertiary neonatal unit over 3.5 years. The unit serves a multicultural population in south-eastern Melbourne and receives outborn infants from other smaller centres. We defined criteria for probable culture-negative meningitis *a priori*, based on data from Garges<sup>9</sup> and Kestenbaum<sup>12</sup>, and our experience that the majority of meningitis diagnoses are made on the basis of CSF pleocytosis (rather than positive bacterial culture) due to prior antibiotic administration. A range of *a priori* definitions for probable culture-negative meningitis were used; none improved the test performance of CRP or ITR.

We acknowledge some limitations of this retrospective single-centre study, including possible differences in defining and managing neonatal infection in other centres, which may limit generalisability. Analogous studies in other settings are warranted. We were unable to use culture-proven meningitis as the sole outcome measure, as this is now rare in most neonatal units, where empiric antibiotics are often commenced prior to a LP being performed;<sup>13, 14</sup> intravenous antibiotics rapidly sterilise the CSF.<sup>15</sup> We therefore used various definitions of CSF pleocytosis as a surrogate for meningitis, but the sensitivity and specificity of these definitions for actual meningitis in our population are impossible to establish. Almost half of infants had traumatic CSF samples; there is no consensus in the literature regarding interpretation of traumatic samples. We used published data<sup>11</sup> to define O:P ratios to categorise traumatic CSF samples. Most of CSF samples were obtained within 48 hours of birth (to exclude early onset meningitis). We are aware of maturational effect of postnatal age on the normative values of WCC in the CSF of newborn infants<sup>16</sup>, however, as most LPs were performed shortly after birth, we lacked power to perform additional analysis by postnatal age. As we did not obtain patient-level clinical data, we could not determine the

timing of the LP relative to commencing antibiotics. However in our unit and elsewhere, pleocytosis in a CSF sample obtained after antibiotics are commenced is often used to determine the need for a treatment course of antibiotics for presumed meningitis. Therefore the findings have real-world clinical applicability in informing criteria on which the decision to perform an LP are made.

Finally, the clinical practice in our unit during the study period was to perform an LP on clinical grounds, a positive blood culture, or if the CRP was >25-30 mg/L. We therefore cannot comment on the incidence of meningitis or CSF pleocytosis in infants not meeting these criteria; such a study would require obtaining CSF in infants in whom it was not thought to be clinically warranted, which is unethical. Further studies that investigate the predictors of meningitis and significant CSF pleocytosis in infants selected on blood culture positivity or clinical grounds alone (but not on CRP or ITR) may partially address this issue.

### **Relationship to prior literature**

Our study is the first to assess the relationship between CRP and ITR and neonatal meningitis.

### **Unanswered questions**

Further studies should address what clinical and possibly laboratory criteria are predictive of abnormal CSF if the decision to perform a LP is made on clinical grounds and/or a positive blood culture. A prospective study with CSF obtained prior to antibiotics would define meaningful thresholds for CSF pleocytosis and interpretation of traumatic LP. Such studies would be difficult to perform in practice as antibiotics are often commenced prior to the decision to perform an LP.

### **Conclusions**

Peripheral inflammatory markers, CRP and ITR, either individually or in combination, have poor test performance in diagnosing culture-positive and probable culture-negative meningitis. The decision to perform a LP should be more focused on clinical grounds and/or a positive blood culture and less on inflammatory or haematological markers in isolation.

## **ACKNOWLEDGEMENTS**

### **Financial disclosure statement**

The authors have no financial relationships relevant to this article to disclose

### **Funding**

Study authors are clinical employees of Monash Health. DPB is an NHMRC Senior Research Fellow and NHFA Honorary Future Leader Fellow. This study required no specific funding support.

### **Potential conflicts of interest**

The authors have no conflicts of interest relevant to this article to disclose

### **Competing interests**

None

## REFERENCES

- [1] Bortolussi R, Krishnan C, Armstrong D, Tovichayathamrong P. Prognosis for survival in neonatal meningitis: clinical and pathologic review of 52 cases. *Canadian Medical Association journal*. 1978; **118**: 165-168.
- [2] Klinger G, Chin CN, Beyene J, Perlman M. Predicting the outcome of neonatal bacterial meningitis. *Pediatrics*. 2000; **106**: 477-482.
- [3] Potgieter S, Dimin S, Lagae L, et al. Epidermoid tumours associated with lumbar punctures performed in early neonatal life. *Developmental medicine and child neurology*. 1998; **40**: 266-269.
- [4] Kalay S, Öztekin O, Tezel G, Demirtaş H, Akçakuş M, Oygür N. Cerebellar Herniation after Lumbar Puncture in Galactosemic Newborn. *AJP Reports*. 2011; **1**: 43-46.
- [5] Patrick SW, Schumacher RE, Davis MM. Variation in lumbar punctures for early onset neonatal sepsis: a nationally representative serial cross-sectional analysis, 2003-2009. *BMC pediatrics*. 2012; **12**: 134.
- [6] Joshi P, Barr P. The use of lumbar puncture and laboratory tests for sepsis by Australian neonatologists. *Journal of paediatrics and child health*. 1998; **34**: 74-78.
- [7] Polin RA, Committee on F, Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. *Pediatrics*. 2012; **129**: 1006-1015.
- [8] Caffrey Osvald E, Prentice P. NICE clinical guideline: antibiotics for the prevention and treatment of early-onset neonatal infection. *Archives of disease in childhood Education and practice edition*. 2014; **99**: 98-100.
- [9] Garges HP, Moody MA, Cotten CM, et al. Neonatal meningitis: what is the correlation among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters? *Pediatrics*. 2006; **117**: 1094-1100.
- [10] Stoll BJ, Hansen N, Fanaroff AA, et al. To tap or not to tap: high likelihood of meningitis without sepsis among very low birth weight infants. *Pediatrics*. 2004; **113**: 1181-1186.
- [11] Greenberg RG, Smith PB, Cotten CM, Moody MA, Clark RH, Benjamin DK, Jr. Traumatic lumbar punctures in neonates: test performance of the cerebrospinal fluid white blood cell count. *The Pediatric infectious disease journal*. 2008; **27**: 1047-1051.
- [12] Kestenbaum LA, Ebberson J, Zorc JJ, Hodinka RL, Shah SS. Defining cerebrospinal fluid white blood cell count reference values in neonates and young infants. *Pediatrics*. 2010; **125**: 257-264.
- [13] Nigrovic LE, Malley R, Macias CG, et al. Effect of antibiotic pretreatment on cerebrospinal fluid profiles of children with bacterial meningitis. *Pediatrics*. 2008; **122**: 726-730.
- [14] Geiseler PJ, Nelson KE, Levin S, Reddi KT, Moses VK. Community-acquired purulent meningitis: a review of 1,316 cases during the antibiotic era, 1954-1976. *Reviews of infectious diseases*. 1980; **2**: 725-745.
- [15] Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics*. 2001; **108**: 1169-1174.
- [16] Connell T, Curtis N. How to Interpret a CSF—The Art and the Science. In: Pollard AJ, Finn A, eds. *Hot Topics in Infection and Immunity in Children II*. Springer US, Boston, MA, 2005; 199-216.

	All infants	Culture proven meningitis †	Culture negative probable meningitis				Sub-total	Non-meningitis ‡
			Atraumatic CSF WCC e 100 /mm <sup>3</sup>	Atraumatic CSF WCC 20-99 /mm <sup>3</sup>	Traumatic OP ratio e 10	Traumatic CSF WCC e 100 /mm <sup>3</sup>		
Male, no (%)	471 (62)	7 (70)	1 (100)	6 (50)	2 (29)	35 (69)	44 (62)	420 (62)
Gestation median (IQR)	38.4 (30.0-40.3)	26.3 (24.3-28.1)	27.4	37.4 (29.9-39.9)	29.6 (28.9-39.6)	30.6 (28.0-38.6)	30.6 (28-39.6)	38.7 (30.6-40.4)
Birth weight(g) (IQR)	2940 (1330-3560)	880 (770-1300)	1110	2810 (1030-2890)	1170 (1160-3040)	1490 (1240-3380)	1490 (1130-3050)	3010 (1440-3600)
Age								
< 3 days no. (%)	475 (63)	0 (0)		4 (33)	5 (71)	40 (78)	49 (69)	426 (63)
3-30 days no. (%)	236 (31)	7 (70)	1 (100)	6 (50)	2 (29)	9 (18)	18 (23)	211 (31)
1-6 months no. (%)	46 (6)	3 (30)		2 (17)		2 (4)	4 (6)	39 (6)
CRP median (IQR)	37.7 (24.3-57.3)	33.7 (21.2-63.1)	74.1	37.4 (30.6-55.2)	4.2 (1.2-37.8)	28.2 (8.2-56.9)	30.6 (8.2-55.7)	38.2 (25.3-57.4)
ITR median (IQR)	0.28 (0.14-0.46)	0.39 (0.22-0.61)	0.60	0.31 (0.07-0.48)	0.33 (0.07-0.45)	0.37 (0.25-0.55)	0.34 (0.19-0.53)	0.27 (0.13-0.45)
pWCC median (IQR)	15.1 (9.9-20.6)	16.3 (8.8-20.3)	8.5	16.1 (13.7-19.9)	10.5 (8.3-13.4)	17.8 (14.1-25.8)	17.0 (12.4-20.9)	15.0 (9.8-20.5)
pNeut median (IQR)	5.8 (3.3-9.5)	5.8 (1.5-8.4)	2.4	5.7 (3.7-9.5)	4.0 (3.3-5.5)	6.7 (3.1-9.9)	5.7 (3.3-9.4)	5.9 (3.3-9.7)
Blood culture pos. no. (%)	117 (16)	6 (60)	1 (100)	4 (33)	1 (14)	6 (12)	12 (17)	99 (15)
Total samples (n)	757	10	1	12	7	51	71	676

Table 1: Table of baseline characteristics for infants by meningitis category.

*CSF indicates cerebrospinal fluid; WCC white cell count; no. Number; pWCC peripheral white cell count, pNeut peripheral neutrophil count, IQR interquartile range. P values in culture proven meningitis column are comparing culture proven meningitis vs all culture-negative. P values in total probable culture-negative column are comparing culture-positive and culture-negative meningitis vs non-meningitis. FBE full blood examination*

† 1 infant did not have FBE or CRP measurements

‡ 5 infants were missing both CRP and ITR, 2 infants were missing CRP only

	Meningitis	Probable culture negative meningitis	Non-meningitis	
CRP < 30	4	35	226	265
CRP e 30	5	36	443	484
	9	71	669	749 †

	Meningitis	Probable culture negative meningitis	Non-meningitis	
ITR < 0.2	2	18	248	268
ITR e 0.2	7	53	423	483
Total	9	71	671	751 ‡

Table 2: Contingency 2x3 table for cut-offs of C-reactive protein 30 mg/L and for immature-to-total neutrophil ratio 0.2.

*CRP, C-reactive protein; ITR, immature-to-total neutrophil ratio † 8 infants did not have CRP measurements ‡ 6 infants did not have ITR measurements*

## FIGURE LEGENDS

Figure 1: Flowchart for definitions of meningitis.

Figure 2. Study flow diagram

Figure 3: C-reactive protein and immature-to-total neutrophil ratio receiver operating characteristic curves and likelihood ratios. CRP indicates C-reactive protein; ITR immature-to-total neutrophil ratio, LR+ positive likelihood ratio, LR- negative likelihood ratio, CI confidence interval, AUC area under the receiver operating characteristic curve

## Learning points

What is already known about this topic

- There is no consensus as to when lumbar puncture should be performed in those with suspected neonatal sepsis and practice varies

What this study adds

- Commonly used inflammatory markers – C-reactive protein and immature to total neutrophil ratio – are poor predictors of neonatal meningitis and pleocytosis and unhelpful in guiding the decision to perform a lumbar puncture
- The decision to perform a lumbar puncture should be based on positive blood culture and clinical grounds

Inflammatory marker and immature neutrophil ratio have no utility in guiding lumbar puncture in suspected neonatal sepsis

Goldfinch C<sup>1</sup>, Korman T<sup>2</sup>, Kotsanas D<sup>2</sup>, Burgner DP<sup>3,4,5,6</sup>, Tan K<sup>1,5,7</sup>

1. Monash Newborn, Monash Children's Hospital, Melbourne, Australia
2. Department of Infectious Diseases, Monash Health, Melbourne, Australia
3. Paediatric Infectious Diseases, Monash Children's Hospital, Melbourne, Australia
4. Murdoch Childrens Research Institute, Melbourne, Australia
5. Department of Paediatrics, Monash University, Melbourne, Australia
6. Department of Paediatrics, University of Melbourne, Melbourne, Australia
7. The Ritchie Centre, PHI-Monash Institute of Medical Research, Melbourne, Australia

### **Original article**

### **Corresponding author**

Full name: Christopher Goldfinch

Email: cgo@live.com.au

Postal address:

Monash Newborn

Monash Medical Centre

246 Clayton Road

Clayton

VIC 3168

Australia

Telephone: 0432368489

### **Ethical approval**

Granted by Quality Unit of the Monash Health Human Research Ethics Committee.

### **Abbreviations**

LP - lumbar puncture

CRP - C-reactive protein

ITR - immature-to-total neutrophil ratio

O:P ratio - observed to predicted ratio of cerebrospinal fluid white cells

WCC - white cell count

RCC - red cell count

ROC curve - receiver operating characteristic curve

IQR - interquartile range

### **Contributors**

CG, KT, and DB conceived the study. CG performed data collection and analysis and wrote the first draft of the manuscript. KT and DB provided input at all stages and critically reviewed all drafts. TK and DK assisted in data extraction and interpretation. All authors have approved the submitted manuscript.