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Procalcitonin as a Serum Biomarker for Differentiation of Bacterial Meningitis From Viral Meningitis in Children: Evidence From a Meta-Analysis

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Abstract

Several studies have explored the use of serum procalcitonin (PCT) in differentiating between bacterial and viral etiologies in children with suspected meningitis. We pooled these studies into a meta-analysis to determine the PCT diagnostic accuracy. All major databases were searched through March 2015. No date or language restrictions were applied. Eight studies (n = 616 pediatric patients) were included. Serum PCT assay was found to be very accurate for differentiating the etiology of pediatric meningitis with pooled sensitivity and specificity of 0.96 (95% CI = 0.92-0.98) and 0.89 (95% CI = 0.86-0.92), respectively. The pooled positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio (DOR), and area under the curve (AUC) for PCT were 7.5 (95% CI = 5.6-10.1), 0.08(95% CI = 0.04-0.14), 142.3 (95% CI = 59.5-340.4), and 0.97 (SE = 0.01), respectively. In 6 studies, PCT was found to be superior than CRP, whose DOR was only 16.7 (95%CI = 8.8-31.7). Our meta-analysis demonstrates that serum PCT assay is a highly accurate and powerful test for rapidly differentiating between bacterial and viral meningitis in children.

Keywords

C-reactive protein (CRP), bacterial meningitis, viral meningitis, children, diagnosis, meta-analysis

Introduction

Bacterial meningitis (BM) in children is a severe, life-threatening illness that requires rapid diagnosis and treatment to decrease the mortality rate and potential neurological sequelae.[1] Clinically, it is often difficult to differentiate between bacterial and viral etiologies of meningitis. Therefore, there is a significant need for a test with a near 100% sensitivity and a high enough specificity to allow for this differentiation.[1]

While lumbar puncture and cerebrospinal fluid (CSF) analysis is the gold standard, supplementary biomarker tests such as C-reactive protein (CRP) and white blood cell count (WBC) are also used clinically. Unfortunately, these tests confer a suboptimal sensitivity and can lead to the initiation of unnecessary empirical antibiotic therapy and hospitalization in patients with self-limiting viral meningitis (VM).[1]

Recently, procalcitonin (PCT) has emerged as a potential new biomarker to replace traditional markers of bacterial infection such as CRP.[2] Procalcitonin is a 116–amino acid calcitonin precursor peptide, which over the recent years has generated widespread interest due to its production by extra-thyroidal tissues in the course of a bacterial infection. Through a hormokine mechanism, proinflammatory mediators such as tumor necrosis factor-α and interleukin-6 cause overexpression of the CALC-1 gene in parenchymal tissues, resulting in a dramatic increase in serum PCT levels.[3]

Interestingly, a similar increase in PCT release is not observed in the course of viral infections. A possible mechanism for this discrepancy was put forth by Linscheid et al[4] who proposed that interferon-γ release in viral infections actually attenuates the release of PCT.

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This incongruence of release forms the basis for the application of PCT as a biomarker to differentiate between viral and bacterial causes of meningitis.

In the emergency setting of bacterial meningitis, the traditional marker CRP has several potentially deleterious limitations. Its delayed elevation, 2 to 8 hours later than PCT elevation,[5] results in potentially fatal false negative tests, early in the disease course.[6-9] Additionally, studies have shown that CRP can be elevated in viral infections,[10,11] further limiting its usefulness as a biomarker to rapidly distinguish between bacterial and viral infections.

The diagnostic accuracy of PCT in differentiating between bacterial and viral meningitis in pediatric populations has been evaluated in several studies.[1,12-18] The results among these studies have varied with sensitivities ranging from 87.5%[17] to 100%[16] and specificities ranging from 66%[12] to 100%,[18] yet no consensus has been reached on the clinical usefulness of PCT. Our aim was to analyze the results from all available studies to determine the true diagnostic accuracy and power of PCT in distinguishing between bacterial and viral meningitis in children, and its potential clinical use.

Methods

Search Strategy

We performed a literature search through March 1, 2015 of the PubMed, EMBASE, Science Direct, Scopus, Web of Science, and Cochrane Library, to identify eligible studies for the meta-analysis. The search strategy was individually adjusted to each of the electronic databases. The search terms included meningitis, meningism, procalcitonin, PCT, S-PCT, and ProCT. No date or language restrictions were set. The references of relevant articles were also searched to ensure identification of all eligible studies. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were strictly followed during the search process and throughout the entire meta-analysis.

Selection of Studies

Articles were considered eligible for inclusion in the meta-analysis if they (1) investigated the diagnostic accuracy of PCT to differentiate between bacterial and viral meningitis in children, (2) measured the serum level of PCT on admission, and (3) reported data necessary to construct 2 × 2 tables (true positives, false positives, false negatives, true negatives). Studies were excluded (1) if they studied exclusively adult populations, (2) if they reported incomplete data or did not provide data necessary to construct 2 × 2 tables, (3) if they had a poor methodological quality, or (4) if they only measured the cerebrospinal fluid levels of PCT. Conference and poster abstracts, case reports, and letters to the editors, were reviewed, but not included in the meta-analysis. Each full-text article was independently assessed by 3 authors (BMH, JV, and PKR) for eligibility in the meta-analysis. Any disagreements during the eligibility processes were settled by a consensus among the authors. When necessary, authors were contact by email for further information regarding the study in question or to request additional data. Articles in languages not spoken by the authors were translated by medical professionals, who are fluent in both English and the language of the article, from their original text into English for further eligibility assessment.

Data Extraction

Data were independently extracted by 2 authors (JV and JR) from the included studies. The data extracted included sample size, mean age, age range, PCT and CRP cutoffs, sensitivity, specificity, PCT assay method, time of measurement, serum levels of PCT and CRP at admission and after treatment, and the study definitions of BM and VM. The authors then constructed 2 × 2 tables to calculate values of true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN). In studies which reported diagnostic data on multiple cutoffs, the cutoff with the highest Youden index score was pooled into the meta-analysis.[19] Authors were contacted by email in the event of discrepancies in the data.

Quality Assessment

The Quality Assessment of Diagnostic Accuracy Studies–2 (QUADAS-2), an evidence-based quality assessment tool was used by 2 authors (JV and JR) to assess the methodological quality of the included studies. The QUADAS-2 assesses risk of bias in four domains (patient selection, index test, reference standard, and flow and timing) and applicability in 3 domains (patient selection, index test, and reference standard) by the use of signaling questions.[20] Each domain was ranked as high risk, unclear risk, or low risk by 2 independent reviewers (JR and JV).

Statistical Analysis

Statistical analysis was performed in MetaDiSc 1.4 by BMH using a random-effects model to calculate pooled sensitivities, specificities, positive likelihood ratio (LR+), negative likelihood ratio (LR−) and diagnostic

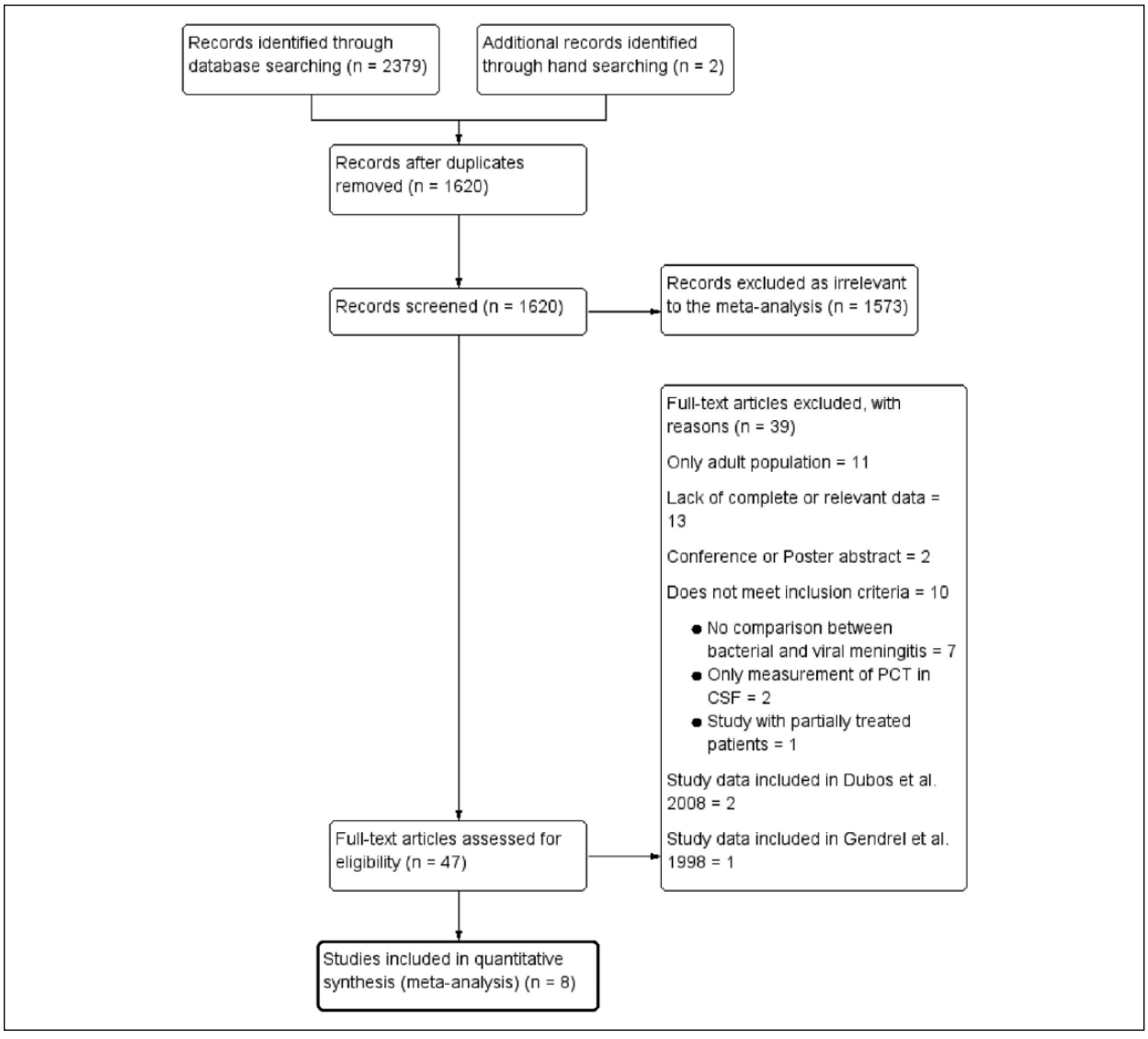

Figure 1. Flowchart of study identification, evaluation and inclusion in the meta-analysis.

odds ratios (DOR). Summary receiver operating characteristic (SROC) curves were formulated and area under the curve (AUC) and index Q* (the point on the SROC where sensitivity and specificity are equal) were calculated to assess overall diagnostic accuracy. For comparing serum PCT levels or CRP levels between admission and 3 days after the initiation of antimicrobial therapy, an effect size was measured by standardized mean difference (SMD) calculated using RevMan 5.3 and interpreted by a set scale (<0.40 = small, 0.40-0.70 = moderate and >0.70 = large).

Higgin's I^2 test was used to assess heterogeneity among the studies with values of 25%, 50%, and 75%, indicating low, moderate, and high degrees of heterogeneity, respectively. Threshold effect was examined using Spearman correlation coefficient, with a P value <.05 being considered significant.[21] An asymmetrical funnel plot was used to assess the potential for publication bias.

Results

Study Identification

The study identification process is summarized in Figure 1. Initially, we identified 2,379 manuscripts through database searching. A further 2 articles were added by reference searching. After screening and duplicate removal, a total of 47 articles were assessed for eligibility using their full-texts. Of these, 39 articles were removed, leaving a total of 8 articles that were deemed eligible and included

in the meta-analysis. Two studies[22,23] were excluded as their data were expanded and included in the study by Dubos et al.[1] The study by Gendrel et al[24] was excluded as there was patient overlap with the study by Gendrel et al in 1998.[14] The study by Prasad et al[25] was deemed ineligible because their bacterial meningitis group included partially treated patients.

Study Characteristics and Quality Assessment

The characteristics for included studies in this meta-analysis is summarized in Table 1. A total of 8 studies (n = 616 patients), 6 prospective and 2 retrospective were included in the study. The studies demonstrated a wide geographical distribution (Egypt, France, China, Turkey, Saudi Arabia, Poland, Spain, and Switzerland). Of the included studies, 6 were in English, 1 in Chinese,[16] and 1 in French.[14] The mean age in the studies ranged from 2.3 to 6.0 years.

All included studies measured serum PCT at admission in patients with suspected meningitis to distinguish between bacterial and viral etiologies. Studies demonstrated a variable cutoff range for PCT between 0.2 ng/mL[14] and 3.3 ng/mL[17] with all studies using the LUMItest PCT assay (BRAHMS Diagnostika, Berlin, Germany) as their testing method.

All of the included studies confirmed the diagnosis of BM using at least one of the following methods: direct CSF examination, gram staining, positive bacterial culture, latex agglutination, or polymerase chain reaction (PCR). The definition of viral meningitis varied between studies. Two studies confirmed diagnosis of VM using viral culture, serological testing, or reverse transcriptase PCR.[12,14] The rest of the included studies defined the diagnosis of VM based on typical CSF findings such as pleocytosis with lymphocytic predominance and normal glucose levels, with the exceptions of studies by Dubos et al,[1,13] which defined VM as acute onset of meningitis with the absence of BM criteria.

The reported sensitivities of the studies measuring PCT ranged from 87.5%[17] to 100%[16] and specificities ranged from 66%[12] to 100%.[14] Seven of the 8 studies also measured CRP as a biomarker, with cutoff values ranging from 1 mg/dL[15] to 20 mg/dL.[12] The reported sensitivity of CRP ranged from 76%[12] to 100%[16] and specificity ranged from 66.7%[1] to 91.3%.[16] Further information on the included studies, including the data resulting from construction of 2 × 2 tables, can be found in Appendix A.

The included quality assessment using the QUADAS-2 tool is summarized in Figure 2.

Diagnostic Accuracy of Procalcitonin

Our results found PCT to be a very sensitive indicator of BM, with pooled sensitivity of PCT equal to 0.96 (95% CI = 0.92-0.98) (Figure 3A). PCT was found to be a more sensitive than specific marker of BM, with a pooled specificity equal to 0.89 (95% CI = 0.86-0.92) (Figure 3B). The LR+ and LR− were 7.5 (95% CI = 5.6-10.1) and 0.08 (95% CI = 0.04-0.14), respectively (Figure 4A and B).

Procalcitonin was found to be a very powerful diagnostic test, with a DOR of 142.3 (95% CI = 59.5-340.4) (Figure 4C). No significant heterogeneity was found among the studies for pooled DOR (I^2 = 17.9%). A SROC was constructed to measure the AUC and index Q*, which were calculated to be 0.97 (SE = 0.01) and 0.91 (SE = 0.02), respectively (Figure 4D).

Diagnostic Accuracy of C-Reactive Protein

Six studies (n = 541 patients)[1,12,13,15-17] compared the diagnostic accuracy of CRP with PCT. CRP was found to be a significantly less sensitive marker of BM as compared with PCT. Pooled sensitivity for CRP was only 0.70 (95% CI = 0.64-0.76) as compared with 0.96 for PCT (Figure 5A). Pooled specificity was not significantly different from PCT at 0.83 (95% CI = 0.79-0.87) (Figure 5B). Pooled LR+ and LR− of CRP were 5.0 (95% CI = 2.7-9.1) and 0.27 (95% CI = 0.12-0.63), respectively. The superiority of PCT was demonstrated in the DOR, in which the DOR of CRP was 16.7 (95% CI = 8.8-31.7) as compared with the 142.3 of PCT. Only very low heterogeneity was detected for CRP (I^2 = 24.1%). SROC was generated and AUC was calculated to be 0.86 (SE = 0.02) and the index Q* to be 0.79 (SE = 0.02).

Heterogeneity and Threshold Effect

Only mild, insignificant heterogeneity was detected for pooled DORs for both PCT (I^2 = 17.9%) and CRP (I^2 = 24.1%). To explore if these mild differences were due to the threshold effect, Spearmen correlation coefficient was calculated for PCT and found to be nonsignificant (R = −0.214, P = .61). Subgroup analysis was performed for 5 studies (n = 473 patients),[1,13,15,16,18] which used a common serum PCT cutoff of 0.5 ng/mL. The pooled sensitivity of 0.97 (95% CI = 0.93-0.99) and the pooled specificity of 0.88 (95% CI = 0.84-0.92), did not change significantly from the general analysis. However, diagnostic odds ratio did increase to 189.8 (95% CI = 69.1-521.4) with no detectable heterogeneity (I^2 = 0.0%). We suspect then that the insignificant heterogeneity may have been caused by the different cutoffs used among the included studies.

Procalcitonin Subgroup Analysis Based on study definition of Viral Meningitis

Subgroup analysis was performed based on the definition of VM used in the individual studies. In a subgroup

Table 1. Characteristics of Studies Included in the Meta-Analysis.[a]

Study	Population	Design	Mean Age [Range] (Years)	Cases (n)		Biomarkers Tested	Cutoff [PCT (ng/mL) CRP (mg/dL)]	Sensitivity (%)	Specificity (%)	TP (n)	FP (n)	FN (n)	TN (n)
				BM	VM								
Alkholi et al (2011)[12]	Egyptian	Prospective	5 [0.33-12]	20	20	PCT	**2**	**100**	**66**	**20**	**7**	**0**	**13**
							10	88	84	18	3	2	17
						CRP	20	76	80	15	4	5	16
Dubos et al (2006)[13]	French	Retrospective	4.6 [0.2-14.9]	21	146	PCT	**0.5**	**89**	**89**	**19**	**16**	**2**	**130**
							0.2	100	31	96	0	70	32
						CRP	2	90	71	19	2	42	104
Dubos et al (2008)[1]	French, Polish, Swiss, Spanish, Turkish	Retrospective	3.2 [0.1-14]	96	102	PCT	**0.5**	**99**	**83**	**95**	**17**	**1**	**85**
						CRP	2	83.2	66.7	79	34	16	68
Gendrel et al (1998)[14]	French	Prospective	3.2 [0.16-13]	23	51	PCT	**0.2**	**100**	**100**	**23**	**0**	**0**	**51**
							0.5	94	100	22	0	1	51
Ibrahim et al (2011)[15]	Egyptian	Prospective	4.3 [0.16-10]	18	20	PCT	**0.5**	**95**	**94**	**17**	**1**	**1**	**19**
						CRP	1	80	90	14.4	2	3.6	18
Liu et al (2006)[16]	Chinese	Prospective	2.3 [0.33-10]	18	23	PCT	**0.5**	**100**	**91.3**	**18**	**2**	**0**	**21**
							0.445	100	87	18	3	0	20
							1.05	100	91.3	18	2	0	21
							2	94.4	91.3	17	2	1	21
							4.06	88.9	95.7	16	1	2	22
							4.83	77.8	95.7	14	1	4	22
							8.395	77.2	100	14	0	4	23
						CRP	2.81	100	91.3	18	2	0	21
Mayah et al (2013)[17]	Egyptian, Saudi Arabian	Prospective	6.05 [0.58-13]	26	32	PCT	**3.3**	**87.5**	**88.6**	**23**	**4**	**3**	**28**
						CRP	2.9	81.2	80	21.1	6.4	4.9	25.6
Onal et al (2008)[18]	Turkish	Prospective	3.2 [0.25-12]	16	13	PCT	**0.5**	**93.7**	**100**	**15**	**0**	**1**	**13**

Abbreviations: BM, bacterial meningitis; VM, viral meningitis; CRP, C-reactive protein; PCT, procalcitonin; FN, false negative; FP, false positive; TN, true negative; TP, true positive.
[a]Cutoff values for PCT included in the meta-analysis are in boldface.

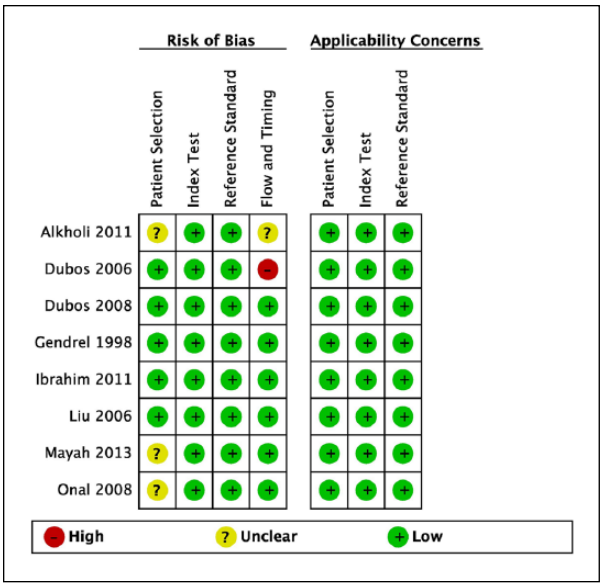

Figure 2. Summary of QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies–2) assessment of included studies.

of 2 studies (n = 124) that confirmed the diagnosis of VM through microbiological methods, the pooled sensitivity, specificity, and DOR for PCT were 0.95 (95% CI = 0.84-0.99), 0.96 (95% CI = 0.88-0.99), and 355.2 (95% CI = 4.1-30524.2, I^2 = 76.3%), respectively.

In a subgroup of 6 studies (n= 531) that defined VM based on either typical CSF changes or acute meningitis in the absence of diagnostic criteria of BM, the pooled sensitivity, specificity, and DOR of PCT were 0.96 (95% CI = 0.92-0.98), 0.88 (95% CI = 0.84-0.91), and 132.17 (95% CI = 56.3-310.4), respectively. While there were slight differences between each subgroup and the general PCT analysis, no difference achieved a level of statistical significance. Furthermore, there were no significant differences between the 2 subgroups themselves.

Publication Bias

The potential for publication bias was assessed through a funnel plot (Figure 6), which revealed asymmetry among the studies. We suspect that this asymmetry was due to the limited number of studies available to include in the meta-analysis.

Change in Serum Levels After Treatment

Three of the included studies (n = 126)[12,15,17] reported the serum levels for both PCT and CRP at admission and 3 days postinitiation of antibiotic therapy in patients with confirmed BM. To study how PCT and CRP levels changed with antibiotic therapy in patients with confirmed bacterial meningitis, we calculated the SMD between admission and 3 days after initiation of antimicrobial therapy for each inflammatory marker. The changes in serum levels after therapy from admission are summarized in Figure 7. For PCT, the serum levels were reduced by at least 50% in each of the 3 studies, 3 days after treatment. The SMD between admission and 3 days posttreatment was 1.05 (95% CI = 0.67-1.42, $P < .00001$), indicating a large and significant effect of the antibiotic therapy. The changes in CRP, however, were highly variable. In 2 of the studies, the level increased at 3 days posttherapy as compared with admission, and in one study it decreased. The SMD for CRP was not statistically significant but indicated no effect after 3 days of antibiotic therapy (−0.12, 95% CI = −0.48 to 0.24, $P = .52$).

Discussion

The use of PCT as a biomarker in pediatric populations is not a completely novel concept. Several studies[26-28] have demonstrated that PCT is a more diagnostically accurate biomarker of serious bacterial infections (SBI) than conventional biomarkers such as CRP and WBC. Recent findings by Mahajan et al[29] indicated the potential use of PCT as an acute-phase biomarker for serious bacterial infections. They reported higher PCT levels (2.9 ± 5.6 ng/mL vs 0.4 ± 0.8 ng/mL, $P = .021$) in young, febrile infants and children with serious bacterial infections than those without it. A review by Pierce et al[30] showed that PCT levels can even be used to tailor antibiotic therapy in children with acute bacterial infection. Another recent review by Reyna-Figueroa et al[31] focused on PCT as a diagnostic biomarker of sepsis in children with cancer, especially if accompanied by neutropenia and pyrexia. These diverse findings encourage the use of PCT as the main biomarker in children with bacterial infections of the central nervous system such as meningitis.

Our results found that PCT is a highly accurate test for the differentiation of bacterial and viral etiologies in pediatric patients with suspected meningitis. With a pooled sensitivity of 0.96 and a pooled LR− of 0.08, a PCT assay is a strongly accurate test for ruling out a bacterial cause of meningitis. These results were far superior to that of CRP, which was found to have a pooled sensitivity and a pooled LR− of only 0.70 and 0.27, respectively.

To measure the value of performing a diagnostic test and the degree to which it modifies the probability of a disease, a likelihood ratio is used. Applying a likelihood ratio after the results of the diagnostic test allows to calculate the posttest probability. It is calculated from the sensitivity and specificity of a test and the values of LR range from 0 to infinity. A value equaling 1, indicates no correlation with a disease and any values above and below 1, increase or decrease the likelihood of having a disease, respectively.[32]

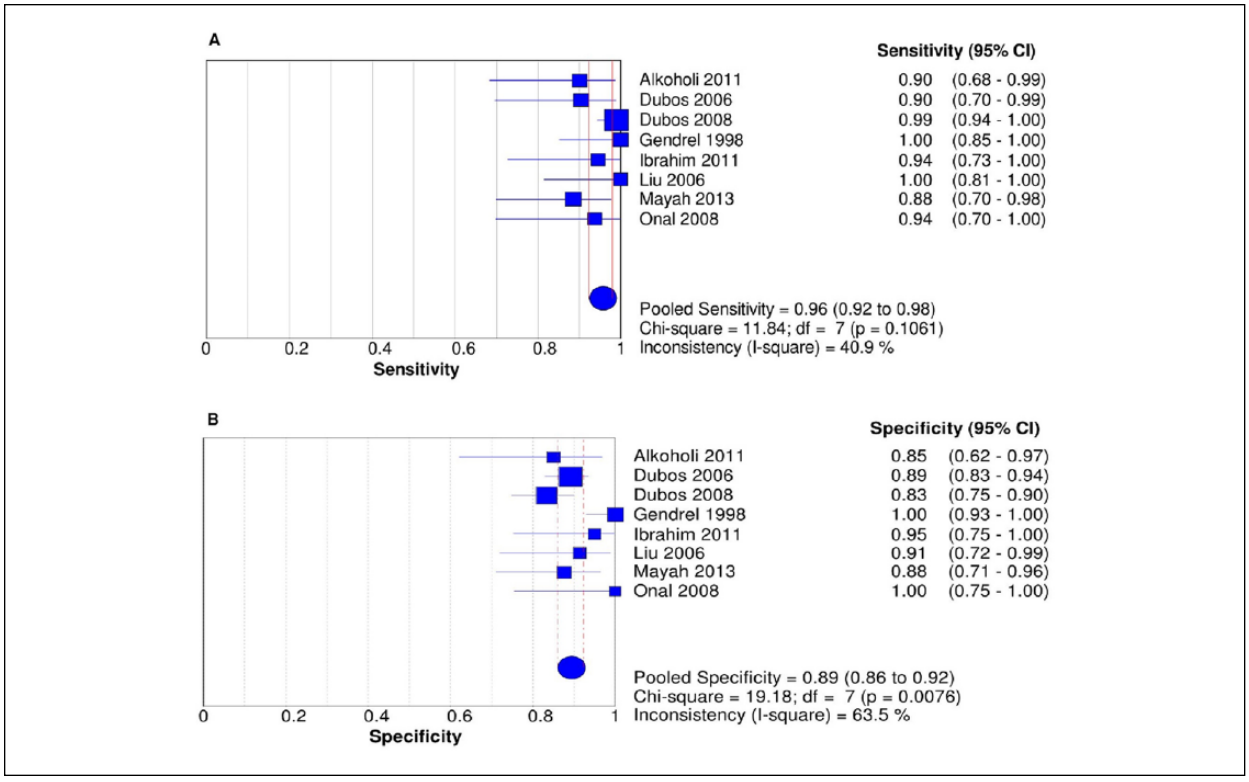

Figure 3. Pooled sensitivity (A) and specificity (B) for serum procalcitonin for the diagnosis of bacterial meningitis in children.

Specificity was found to be more similar between PCT and CRP, with PCT still maintaining superior values (0.89 vs 0.83). However, the superior power of PCT over CRP is reflected in the DOR. Combining sensitivity and specificity leads to the DOR, which is a single measure reflecting the discriminatory effectiveness of a diagnostic test. Ranging from 0 to infinity, higher values represent a greater ability to differentiate between diseased and healthy subjects.[33] The DOR of PCT was 142.3 compared with only 16.7 for CRP, indicating that PCT is a much more clinically effective diagnostic measure than CRP. Overall assessment was reflected in AUC which was 0.97 for PCT assay as compared with 0.86 for CRP.

The PCT cutoffs in the included studies ranged from 0.2 to 3.3 ng/mL. When subgroup analysis was performed on 5 of the 8 studies that used a common PCT cutoff value of 0.5 ng/mL, sensitivity increased by only 1% (from 0.96 to 0.97) and specificity decreased by only 1% (from 0.89 to 0.88). However, we would recommend the use of a 0.5 ng/mL cutoff for PCT, due to the higher power, reflected in the increase in DOR from 142.3 in the general analysis, to 189.8 in the subgroup with the 0.5 ng/mL cutoff.

The hormokine nature of PCT during bacterial infections makes it valuable as a potential biomarker when rapid differentiation between bacterial and viral causes of meningitis is required. Several studies[34-36] have also suggested that quantification of serum PCT levels may correlate with the severity of infections. As such, PCT may be useful as a potential indicator of prognosis in meningitis.[37,38]

In our study, we also attempted to assess how serum levels of PCT and CRP change over the course of treatment in BM, and to gain insight into which marker correlates better with antimicrobial therapy. We found that serum levels of PCT reduced by at least 50% from time of admission to 3 days after the initiation of therapy, and that the effect size of antibiotic therapy measured by SMD for PCT levels was strong. This was in contrast to CRP, whose serum levels varied across the studies, increasing in 2 studies from admission and decreasing in 1 study, and demonstrated no effect of antibiotic therapy. This tendency is supported by Hu et al,[38] who found a significant decline in serum PCT, 3 days after initiation of effective antibiotic therapy. Conversely, in patients with poor clinical improvement, serum PCT did not show a similar marked decrease. The authors also investigated how serum PCT levels reflect disease severity. Their findings showed that higher serum PCT levels correlated with a more severe clinical presentation and a higher mortality.[38] As such, prolonged elevation of serum PCT after administration of antibiotics may reflect poor treatment efficacy, indicating the need

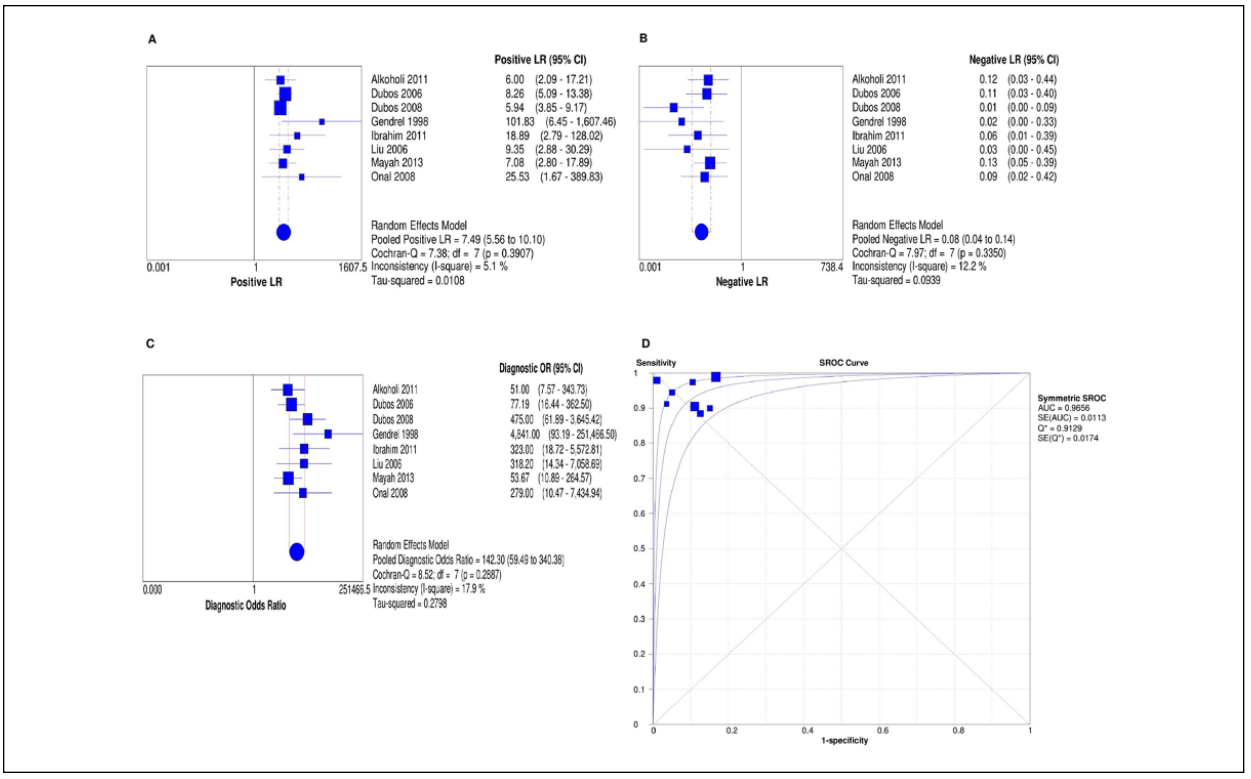

Figure 4. Pooled positive likelihood ratio (LR+) (A), negative likelihood ratio (LR−) (B), diagnostic odds ratio (DOR) (C), and summary receiver operating characteristic (SROC) (D) for serum procalcitonin for the diagnosis of bacterial meningitis in children.

for prompt reevaluation of treatment, to prevent mortality in children with BM. However, further studies are needed to measure how serum PCT levels correlate with the efficacy of antibiotic therapy, and the potential use of serum PCT to evaluate prognosis in patients with BM.

In order for PCT testing to be widely and routinely administered, health care costs must be taken into consideration. Even though CRP is tested using numerous, inexpensive assays costing between US$1 and US$10,[39] a study by Nabulsi et al[40] demonstrated that routine CRP testing inflated hospital bills without affecting the medical decision making.[40] The turnaround time for CRP tests is approximately 50 minutes, which can delay the initiation of immediate intervention.[41]

On the other hand, new PCT assays such as Kryptor, which cost around US$10 and US$40,[39] have a significantly shorter turnaround time of 20 minutes.[42] This provides a significant advantage to clinical usage of PCT, thus allowing for a more rapid and appropriate implementation of treatment, as well as avoiding unnecessary antibiotic therapy and associated costs.

Additionally, serum PCT levels have been demonstrated as unchanging with the administration of nonsteroidal anti-inflammatory drugs and glucocorticosteroids, unlike those of CRP.[43,44] This is especially valuable in the emergency setting of meningitis in children, in which parents may have previously administered nonsteroidal anti-inflammatory drugs to their children prior to admission, for symptomatic relief.

While CSF analysis is considered the gold standard for the diagnosis of BM, PCT has been shown to outperform the traditional markers. In a 2010 retrospective, multicenter, hospital-based cohort study by Dubos et al,[45] the authors demonstrated that 99% of patients with BM had a serum PCT level >0.5 ng/mL. This was superior to the proportion of patients with positive traditional CSF values such as CSF gram staining (75%), CSF protein level >50 mg/dL (88%), CSF protein level >80 mg/dL (77%), and CSF neutrophil count >1000 × 10^6 (53%). These results suggest that PCT may be an accurate marker in patients with suspected meningitis and negative or nonconclusive CSF results. However, further studies are needed to explore the use of PCT as a diagnostic marker in this patient group.

In the study by Onal et al,[18] included in our meta-analysis, PCT had equal specificity to CSF leukocyte count, CSF:blood glucose ratio, and CSF protein level, all of which showed 100% specificity for BM. However, PCT had a sensitivity of 93%, which was higher in comparison with CSF leukocyte count (75%) and CSF protein level

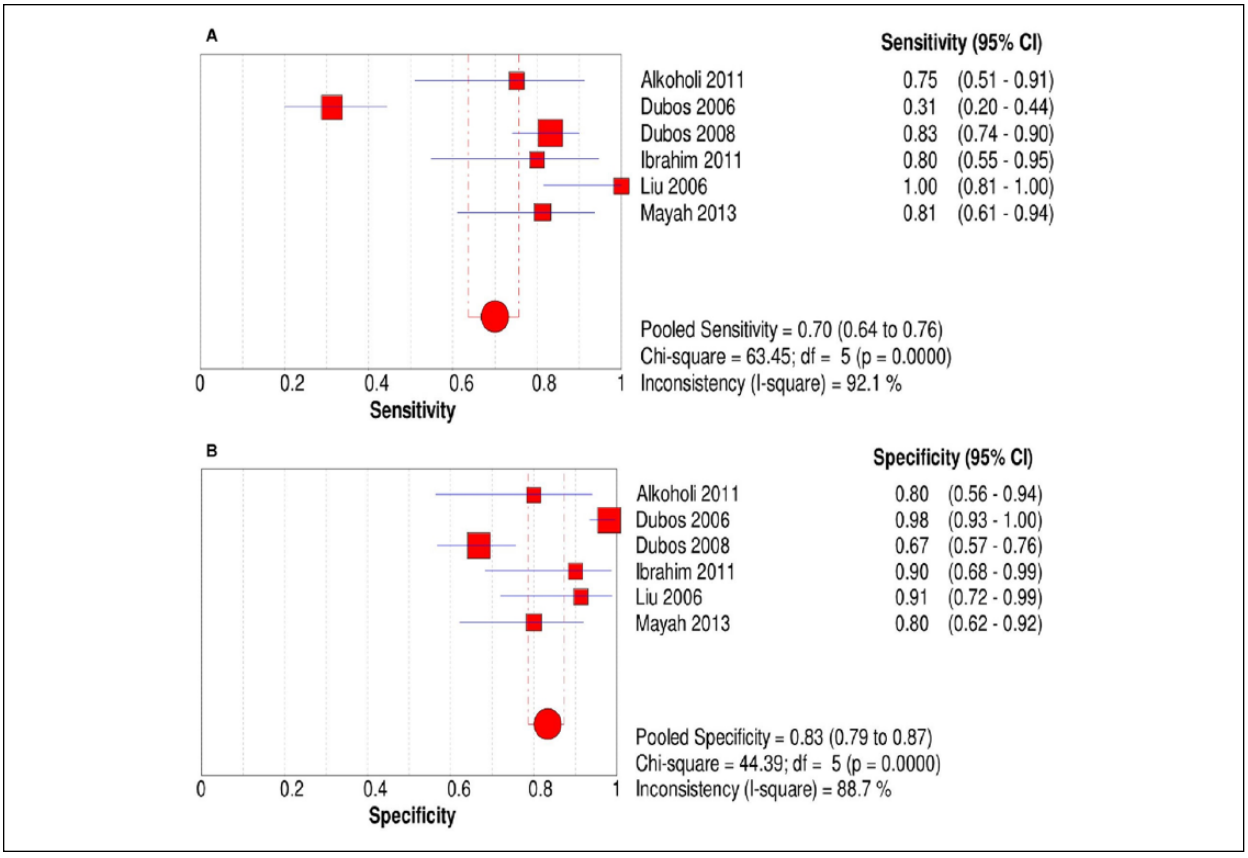

Figure 5. Pooled sensitivity (A) and specificity (B) for C-reactive protein (CRP) for the diagnosis of bacterial meningitis in children.

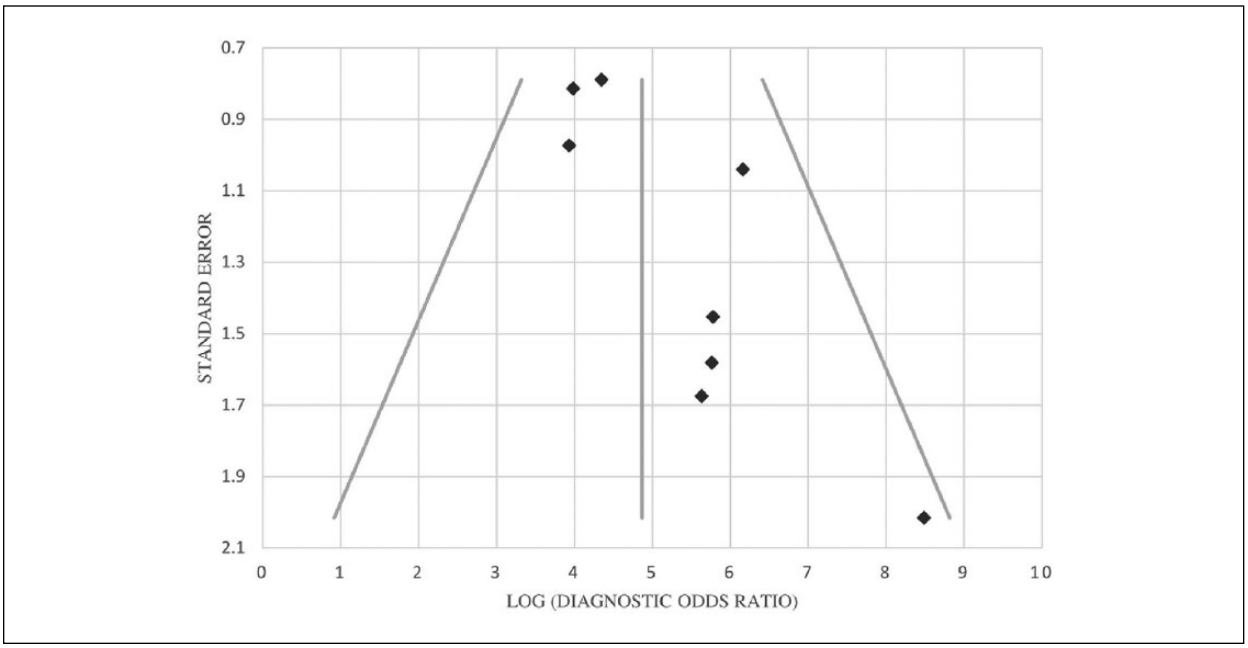

Figure 6. Funnel plot presenting study heterogeneity and potential for publication bias.

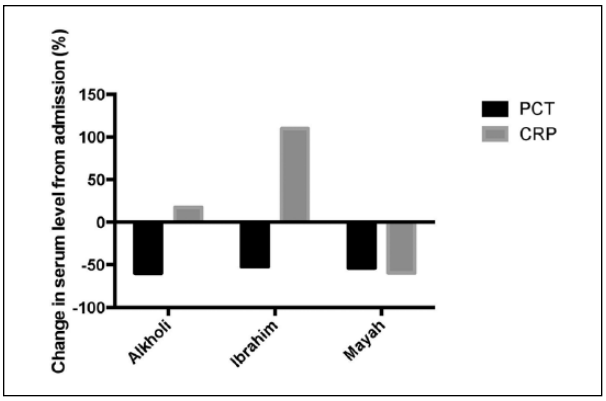

Figure 7. Changes in serum levels of procalcitonin (PCT) and C-reactive protein (CRP) from admission to 3 days after initiation of antimicrobial therapy.

(85.7%), and equal to CSF:blood glucose ratio (93%).[18] The superior sensitivity of PCT as compared to traditional CSF markers of BM, demonstrates its clinical usefulness in collaboration with lumbar puncture for a more accurate diagnosis of the etiology of suspected meningitis.

Bacterial meningitis is not diagnosed using CSF biomarkers in isolation, rather clinicians often first rely on physical signs in order to form a suspicion of the patient's condition. However, these signs do not demonstrate perfect sensitivities and specificities for diagnosing meningitis, and therefore, must be relied on with caution. For example in children, Brudzinski's sign has been shown to have a sensitivity and specificity of 52.6% and 77.5%,[46] Kernig's sign has sensitivities ranging from 9%[47] to 51.4%[46] and specificities ranging from 87%[48] to 100%,[47] and nuchal rigidity has sensitivities ranging from 15%[47] to 64.5%[46] and specificities ranging from 53.5%[46] to 100%.[47] Other nonspecific clinical signs such as headache has a sensitivity and specificity of 76% and 53%, respectively, and photophobia had a very low sensitivity of 28% and specificity of 88%.[48] As such, it is important to take into account the entire clinical picture when formulating a differential diagnosis.

There are a few important limitations of PCT that must be considered when applying the assay clinically. As noted above, due to the decline in PCT levels with antibiotics, PCT may have limited use in children who had recently taken antibiotics prior to presenting with symptoms of meningitis. Another limitation for the use of PCT in suspected meningitis is the presence of other serious bacterial infections such as pneumonia and sepsis. As PCT is raised in most cases of serious bacterial infections, the diagnostic accuracy of PCT is likely limited in children presenting with symptoms of acute meningitis in the presence of other bacterial infections.[28] Furthermore, it likely has a limited ability to differentiate between BM and other causes of acute febrile encephalopathy due a bacterial pathogen, such as a brain abscess.

Our meta-analysis was limited by the small number of studies available for inclusion in the meta-analysis, and the relatively small sample sizes of some of the included studies. This was probably the source of the asymmetry found in our funnel plot to probe publication bias. No other publication bias testing method such as Begg's or Egger's test was performed, as the current tests for publication bias are primarily designed to assess bias in interventional studies.[49,50] As such, these publication bias tests in diagnostic studies, have a low power and can yield seriously misleading results.[49,50] Accurate determinants for assessment of publication bias in diagnostic studies are yet to be determined.[49,50]

However, despite the limitations, no significant heterogeneity was detected between the studies. No significant differences were found between subgroups based on the study definition of VM. Furthermore, the use of the same PCT testing assay by all of the studies also allowed for more accurate pooling of the data. As such, our findings provide strong evidence that PCT is a highly accurate and powerful marker for bacterial meningitis.

With a turnaround time as short as 20 minutes,[42] we recommend that the test be applied clinically by physicians in collaboration with clinical history, physical examination, basic laboratory results, and CSF analysis, to rapidly differentiate between bacterial and nonbacterial meningitis in children. Because of its high sensitivity, it can be used to quickly determine if a bacterial infection is the cause of suspected meningitis, with an accuracy superior to that of most traditional biomarkers. Hence, the clinical implementation of PCT testing may increase the accuracy of meningitis diagnosis, and reduce unnecessary antibiotic therapy and hospitalization costs.

Conclusions

Procalcitonin is a highly accurate and powerful diagnostic serum biomarker that allows for rapid differentiation between bacterial and viral etiologies in children with suspected meningitis. PCT is a superior diagnostic test as compared with CRP for detection of BM. Because of the high sensitivity of PCT assays, we recommend its regular use by physicians in the emergency setting for quickly ruling out BM, and using it to supplement clinical history, physical examination, and CSF analysis for a more accurate diagnosis. As such, PCT may help to reduce unnecessary treatment and hospitalization. Finally, PCT appears to be an effective marker to monitor the efficacy of antimicrobial treatment, and could potentially be used to assess prognosis in children with BM.

Appendix A

Further Information on Studies Included in the Meta-Analysis.

Confirmed bacterial meningitis AND confirmed viral meningitis

Author	Year	Method of BM Confirmation	Method of VM Confirmation	Confirmed BM (cBM) (n=)	cBM: Etiologies	Confirmed VM (cVM) (n=)	cVM: Etiologies	Time of PCT Measurement	PCT Cutoff for cBM (ng/mL)	PCT Sensitivity (%)	PCT Specificity (%)	TP (n=)	FN (n=)	TN (n=)	FP (n=)	Time of CRP Measurement	CRP Cutoff for cBM (mg/dL)	CRP Sensitivity (%)	CRP Specificity (%)	TP (n=)	FN (n=)	TN (n=)	FP (n=)
Alkholi	2011	BM was defined as bacterial according to CSF laboratory findings (increased protein ≥2 g/l, decreased glucose ratio ≤0.4, and leukocyte count ≥1500 × 10/l and polymorph nuclear leukocyte domination), identification of bacterial agents in Gram staining, and/or positive bacterial culture.	It was defined as viral meningitis if the viral culture, serological testing, pleocytosis, or reverse transcriptase polymerase chain reactions were positive, and the bacterial culture was negative.	20	*N. meningitidis* (n = 6), S. pneumoniae (n = 6), *H. influenzae* (n = 4), E. coli (n = 2), P. aeruginosa (n = 1), BM with negative Gram stain (n = 1)	20	Enterovirus (n = 12), Mumps (n = 3), Measles (n = 2), Varicella (n = 2)	Admission	10	88	84	18	2	17	3	Admission	20	76	80	15	5	16	4
Gendrel	1998	Bacterial meningitis was confirmed after bacterial cultures of CSF.	Aseptic meningitis was confirmed if patients had symptoms of meningitis, CSF leukocytes counts of > 20/ uL, and negative bacterial cultures. Viral cultures and PCR were performed for these group of patients.	23	*N. meningitidis* (n = 10), H. influenza (n = 6), S. pneumoniae (n = 5), E. coli (n = 1), L. monocytogenes (n = 1)	51	Enterovirus (n = 4), Adenovirus (n = 2), VZV (n = 1); From PCR, Enterovirus (n = 42)	Admission	0.2	100	100	23	0	51	0								

Confirmed bacterial meningitis AND nonconfirmed viral meningitis with CSF definitions

Author	Year	Method of BM Confirmation	Method of AM Confirmation	Confirmed BM (cBM) (n=)	cBM: Etiologies	AM (n=)	cAM: Etiologies	Time of PCT Measurement	PCT Cutoff for cBM (ng/ml)	PCT Sensitivity (%)	PCT Specificity (%)	TP (n=)	FN (n=)	TN (n=)	FP (n=)	Time of CRP Measurement	CRP Cutoff for cBM (mg/dL)	CRP Sensitivity (%)	CRP Specificity (%)	TP (n=)	FN (n=)	TN (n=)	FP (n=)
Ibrahim	2011	Meningitis was defined as bacterial if the CSF laboratory findings showed: (increased protein >2g/l, decreased glucose ratio <0.4, leukocyte count > 1500 × 106/l and polymorph nuclear leukocyte domination), identification of bacterial agents in gram staining and/or positive bacterial culture	Patients were included in AM group if no bacteria were documented on Gram-stain or bacterial culture of CSF, lymphocyte predominance of CSF cells, reduced protein level, and increased glucose ratio >0.5.	18	*N. meningitidis* (n = 5), S. pneumonia (n = 5), *H. influenzae* type b (n = 4), E. coli (n = 2), S. aureus (n = 2).	20		Admission	0.5	95	94	17	1	19	1	Admission	1	80	90	14	4	18	2
Liu	2006	Bacterial meningitis was confirmed by the presence of clinical signs and symptoms plus one of a) +ve CSF smear or culture or b) negative CSF smear or culture, but positive bacterial culture plus typical changes in CSF i.e. increased protein (>1g/L), increased WBC (1x10^9, mainly neutrophils) and decreased glucose.	Viral meningitis was confirmed by the presence of clinical signs and symptoms plus CT and MRI plus typical changes in CSF (normal or increased number of cells, mainly lymphocytes, normal or increased protein level and normal glucose and chloride levels) plus -ve CSF bacterial culture/smear	18	None given	23		Admission	0.5	100	91.3	18	0	21	2	Admission	2.81	100	91.3	18	0	21	2
Mayah	2013	Children presenting with clinical symptoms of meningitis; fever, headache, stiff neck, bulging fontanelle or mental status changes, CSF with an elevated protein (>100 mg/dl), decreased glucose (<40 mg/dl) or leukocytosis (WBC > 100/ mm3) with at least 80% neutrophils. Identification of bacteria directly by Gram stain smears or cultures from blood or CSF or indirectly by latex agglutination test of CSF	Aseptic meningitis was defined as the presence of acute onset of meningitis symptoms, WBC of > 5/ mm3 of which > 50% were mononuclear/lymphocyte cells with the absence of any bacterial meningitis laboratory criteria.	26	*N. meningitidis* (n = 12), *H. influenzae* type B (n = 8), S. pneumoniae (n = 6)	32		Admission	3.3	87.5	88.6	23	3	28	4	Admission	2.9	81.2	80	21	5	26	6

(continued)

Appendix A (continued)

Confirmed bacterial meningitis AND nonconfirmed viral meningitis with CSF definitions

Author	Year	Method of BM Confirmation	Method of AM Confirmation	Confirmed BM (cBM) (n=)	cBM: Etiologies	AM (n=)	cAM: Etiologies	Time of PCT Measurement	PCT Cutoff for cBM (ng/ml)	PCT Sensitivity (%)	PCT Specificity (%)	TP (n=)	FN (n=)	TN (n=)	FP (n=)	Time of CRP Measurement	CRP Cutoff for cBM (mg/dL)	CRP Sensitivity (%)	CRP Specificity (%)	TP (n=)	FN (n=)	TN (n=)	FP (n=)
Onal	2008	The diagnosis of bacterial meningtis was made according to documentation of infection in CSF culture, CSF pleocytosis > 10 cells/mm3, predominance of PNMs in direct examination of CSF, presence of bacteria in direct examination of CSF, CSF glucose to blood glucose ratio < 0,6, increase in CSF protein, and positivity of bacterial antigen in CSF.	Patients with predominance of lymphocytes in CSF pleocytosis, slight increase in protein, and with normal glucose levels in CSF, no bacteria in direct examination of CSF, and negativity of bacterial antigens were considered to have viral meningitis.	16	None given	13		Admission	0.5	93.7	100	15	1	13	0								

Confirmed bacterial meningitis AND nonconfirmed viral (aseptic) meningitis with only clinical definition

Author	Year	Method of BM Confirmation	Method of AM Confirmation	Confirmed BM (cBM) (n=)	cBM: Etiologies	AM (n=)	cAM: Etiologies	Time of PCT Measurement	PCT Cutoff for cBM (ng/ml)	PCT Sensitivity (%)	PCT Specificity (%)	TP (n=)	FN (n=)	TN (n=)	FP (n=)	Time of CRP Measurement	CRP Cutoff for cBM (mg/dL)	CRP Sensitivity (%)	CRP Specificity (%)	TP (n=)	FN (n=)	TN (n=)	FP (n=)
Dubos	2006	Bacterial meningitis was defined as the acute onset of meningitis as defined earlier, with documented bacterial infection in the CSF (direct examination, culture or latex agglutination) or blood culture.	Aseptic meningitis was defined as the acute onset of meningitis and the absence of any bacterial meningitis criteria.	21	S. pneumoniae (n = 10), *N. meningitidis* (n = 9), H influenzae type b (n = 1), *S. agalactiae* (n = 1)	146		Admission	0.5	89	89	19	2	130	16	Admission	2	90	71	19	42	104	2
Dubos	2008	Bacterial meningitis was defined as the acute onset of meningitis (CSF WBC count >7/μL) and documented bacterial infection in CSF (direct examination, culture, latex agglutination, or polymerase chain reaction) or blood culture.	Aseptic meningitis was defined as the acute onset of meningitis and the absence of any bacterial meningitis criteria	96	N, meningitidis (n = 45), S. pneumoniae (n = 32), *H. influenzae* (n = 7), *S. agalactiae* (n = 4).	102		Admission	0.5	99	83	95	1	85	17	Admission	2	83.2	66.7	79	16	68	34

Abbreviations: BM, bacterial meningitis; VM, viral meningitis; AM, aseptic meningitis; PCT, procalcitonin; CRP, C-reactive protein; TP, true positives; FN, false negatives; TN, true negatives; FP, false positives.

Appendix B

PRISMA 2009 Checklist.

Section/Topic	#	Checklist Item	Reported on Page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3 - 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5 - 6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6

(continued)

Appendix B (continued)

Section/Topic	#	Checklist Item	Reported on Page #
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7 - 8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 - 10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8 - 10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11 - 16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	15 - 16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1, 17

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Author Contributions

Conceived and desgined the experiments: BMH, KAT, JAW.
Performed the experiments: BMH, JR, JV, PKR.
Analyzed the data: BMH, PKR, KAT.
Wrote the paper: BMH, JR, JV, PKR, KAT, JAW.

Declaration of Conflicting Interests

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