5-30-2016

Maternal Inflammatory Markers in the Diagnosis of Chorioamnionitis and Prediction of Neonatal Sepsis in Preterm Pre-Labour Rupture of Membranes: A Systematic Review

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MATERNAL INFLAMMATORY MARKERS IN THE DIAGNOSIS OF CHORIOAMNIONITIS AND PREDICTION OF NEONATAL SEPSIS IN PRETERM PRE-LABOUR RUPTURE OF MEMBRANES; A SYSTEMATIC REVIEW

By

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A dissertation submitted in part fulfilment of the requirements for the degree of
Master of Medicine
In Obstetrics and Gynaecology

Nairobi/Kenya

30 May 2016

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In part fulfilment of the requirements for the degree of
Master of Medicine
In Obstetrics and Gynaecology

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ABSTRACT

Background

There is no consensus on the potential role of inflammatory markers in identifying chorioamnionitis in women with Preterm Pre-labour Rupture of Membranes (PPROM) or in predicting Early Onset Neonatal Sepsis (EONS) in their neonates.

Objectives

To perform a quantitative review on the accuracy of maternal C reactive protein (CRP), Procalcitonin (PCT) and Interleukin 6 (IL6) in the diagnosis of Histological Chorioamnionitis and/or Funisitis (HCA/Funisitis) and their role in the prediction of EONS in PPROM.

Methods

MEDLINE, EMBASE and The Cochrane Library databases were searched from inception to October 2015, for studies where these markers were assessed against a reference standard of HCA/Funisitis or outcome of EONS in PPROM. Two reviewers independently performed screening, data extraction and quality assessments. The Quality Assessment of Diagnostic Accuracy Studies 2(QUADAS-2) and the Quality in Prognostic Studies (QUIPS) tools were used to assess methodological quality. Hierarchical summary receiver operating characteristic (SROC) models were used in the diagnostic review. In the prognostic review, unadjusted Odds Ratios (ORs) were pooled in a random effects meta-analysis.

Results

The diagnostic review included 14 studies reporting 361 episodes (47.4%) of HCA/Funisitis in 761 participants, median prevalence 41% (IQR 36-53). The pooled indices for CRP at the commonest cut-off of 20mg/L (5 studies, 252 participants) were sensitivity 59% (95% CI 48-69), specificity 83% (95% CI 74-89), Likelihood Ratio positive (LR+) 3.45(95% CI 2.24-5.30) and Likelihood Ratio negative (LR-) 0.50(95% CI0.38-0.64). The sensitivity, LR+ and LR- for CRP at all cut-offs (11 studies, 570 participants) and at a selected specificity of 80% were 55%, 2.75 and 0.56 respectively. Indices for IL6 at a specificity of 80% were sensitivity 62%, LR+ 3.1 and LR- 0.48. No pooled indices were derived for PCT as included studies were few.

The prognostic review included 7 studies with 332 participants and 97 episodes of EONS, median prevalence 26% (IQR 26-34). The pooled unadjusted OR for studies evaluating CRP at the commonest cut-off of 10mg/L (4 studies, 161participants) was 2.79 (95%CI 1.33-

5.88, p 0.007). No pooled estimates were obtained for PCT and IL6 as included studies were few. Included studies were mainly prospective cohort design but were of poor quality.

Conclusions

There is insufficient evidence to support use of CRP, PCT or IL6 in maternal blood for the diagnosis of HCA/Funisitis in PPROM and prediction of EONS in PPROM.

Recommendations

We do not recommend the routine use of maternal CRP, PCT or IL6 singly in the management of PPROM. There is need for good quality prospective cohort studies to better assess the role of these biomarkers in PPROM.

LIST OF TERMS AND ABBREVIATIONS USED

Abbreviation	Meaning
AKU	Aga Khan University
CCA	Clinical Chorioamnionitis
CI	Confidence Interval
CRP	C Reactive Protein
EONS	Early Onset Neonatal Sepsis
FN	False Negative
FP	False Positive
HCA	Histological Chorioamnionitis
HCA/Funisitis	Histologic Chorioamnionitis and/or Funisitis
HSROC	Hierarchical Summary Receiver Operating Characteristic
IL6	Interleukin 6
IQR	Inter Quartile Range
LR	Likelihood Ratio
NPV	Negative Predictive Value
NR	Not Reported
OR	Odds Ratio
PCT	Procalcitonin
PPV	Positive Predictive Value
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PPROM	Preterm Pre-labour Rupture of Membranes
PROM	Pre-labour Rupture of Membranes
PROSPERO	International Prospective Register of Systematic Reviews
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies-2
QUIPS	Quality in Prognostic Studies
ROM	Rupture of Membranes
SD	Standard Deviation
SE	Standard Error
SROC	Summary Receiver Operating Characteristic
STARD	Standards for Reporting Diagnostic Accuracy Studies
TN	True Negative
TP	True Positive

ACKNOWLEDGEMENT

I am grateful to my supervisors Dr. Geoffrey Omuse, Dr. Mwaniki Mukaindo and Prof. Marleen Temmerman, whose scholarly advice, help and constant encouragement have contributed significantly to the completion of this study. Dr. Mukaindo and Dr. Omuse also served as independent reviewers in the stages of screening abstracts, reviewing full texts, data extraction and performing quality assessments for the review. I appreciate their assistance in these very time consuming tasks.

I also wish to thank Prof. William Stones and Stella Glasmacher for their advice during the proposal development stage. I thank Alex Maina and Anthony Etyang of KEMRI-Wellcome Trust Research Programme–Kilifi, Laura Hammitt of Johns Hopkins School of Public Health and Nasra Gathoni of Aga Khan University, Nairobi library for assistance in retrieving full texts. Nasra Gathoni also assisted in reviewing the electronic search strategy.

I wish to thank my Dissertation Committee members for their critical input for my study.

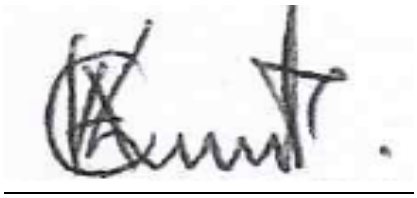
I also wish to thank the management, staff, faculty members, and my fellow residents for their invaluable input and for being a great source of support to me during my study. Special mention to Demetrius Mududa and John Kimani.

I am grateful to my family and friends for their constant support and encouragement throughout the Dissertation development process. Special mention to Anthony Etyang, and Lynda Koech.

Thank you all

DECLARATION

I declare this dissertation does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university and that to the best of my knowledge it does not contain any material previously published or written by another person except where due reference have been made in the text.



(Signature of Candidate)

30 May 2016
Date

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BACKGROUND

Pre-labour Rupture of Membranes (PROM) is defined as rupture of membranes before the onset of uterine contractions. Preterm Pre-labour Rupture of Membranes (PPROM) refers to PROM occurring before $37^{0/7}$ weeks of gestation.[1] It complicates up to 2-3% of pregnancies.[1,2] PPROM and its complications cause several adverse maternal and neonatal outcomes largely due to infection related complications and the additional risk of prematurity related complications to the neonate.[1,3–6]

PPROM is associated with 40% of preterm births.[5] The foetus and the neonate are at greater risk of PPROM related morbidity and mortality than the mother.[7] Outcomes for the neonate are even poorer in the setting of infection related morbidity than they are for a similar uninfected preterm neonate.[8,9]

PPROM and its infectious complications

Infectious complications in PPROM arise from ascending infection by microorganisms in the setting of membrane rupture.[10,11] While intra-amniotic infection may also occur in the presence of intact membranes and via other routes of infection, it is commonest in the setting of PPROM.[11]

The presence of microorganisms in this otherwise sterile compartment triggers a maternal and foetal inflammatory response.[10,12,13] Chorioamnionitis refers to acute inflammation of the amnion and chorion of the placenta.[13–16] Funisitis is said to be present when the inflammatory processes involve the umbilical cord: the umbilical vein, umbilical artery and the Wharton's jelly.[13] A variety of pro-inflammatory and inhibitory cytokines and chemokines are released into the maternal and foetal compartments.[10,17] Resultant inflammation can produce the clinical features of chorioamnionitis and may also lead to prostaglandin release, cervical ripening and membrane injury. This could in turn lead to preterm labour and/or PPROM. Intra-amniotic infection may therefore be both a cause and a consequence of PPROM.[10,18]

Chorioamnionitis is classified as Clinical Chorioamnionitis(CCA) and Histologic Chorioamnionitis(HCA).[14] A diagnosis of CCA is made when specific clinical signs are present. The essential criterion is maternal fever defined in different studies as temperature $\geq$ 37.8^0C(100^0F) or $\geq$ 38.0^0C (100.4^0F).[14,15,19] The presence of risk factors for the disease,

non-specific clinical signs and exclusion of other sources of fever further support a clinical diagnosis. For research purposes, a diagnosis of overt CCA is based on the presence of maternal fever ≥38^{0}C (≥100.4 F) and at least two nonspecific signs: maternal leucocytosis(>15,000cells/mm^3), maternal tachycardia (>100 beats/minute), foetal tachycardia (>160beats/minute), uterine tenderness and foul smelling liquor.[14,16,20,21]

A diagnosis of HCA and funisitis is made upon microscopic examination of the placenta after delivery. HCA is based on the presence of neutrophil infiltrates, necrosis, amnion basement membrane thickening and chorionic micro abscesses[22] and funisitis or presence of neutrophil infiltrates in the umbilical cord vessels or Wharton's jelly. HCA may occur in CCA but has been demonstrated in cases with no clinical features of infection where it is referred to as subclinical chorioamnionitis.[23] Intra-amniotic infection is diagnosed by a positive culture of microorganisms from an appropriately collected sample of amniotic fluid or chorio-amnion.[16]

In the mother, chorioamnionitis predisposes to complications such as endo-myometritis, wound infection, pelvic abscess and septicaemia. It may also rarely cause septic shock, disseminated intravascular coagulopathy and maternal death. It is associated with a 2 to 3 fold increased risk of caesarean section and an increased risk for postpartum haemorrhage.[3,24] In the foetus, chorioamnionitis could lead to systemic infection. Short term complications include pneumonia, meningitis, asphyxia, intra-ventricular haemorrhage, respiratory distress syndrome, septic shock and early onset neonatal sepsis (EONS).[20,24] Neurodevelopmental delay and cerebral palsy are recognised longer term complications. [25,26]

Diagnosis and Management of PPROM / Clinical Pathway

The diagnosis of rupture of membranes is largely clinical. Suggestive maternal history and visualisation of a pool of fluid in the posterior vaginal fornix is usually sufficient to make a diagnosis[1]. Several tests have been used to confirm whether the fluid visualised is indeed amniotic fluid. These tests include the Nitrazine test, the ferning test, tests based on microscopic examination of lanugo hair or foetal epithelial tests and newer rapid tests based on detection of insulin like growth factor binding protein- 1, placental alpha macroglobulin – 1 and other markers.[27] These are not essential for the diagnosis of rupture of membranes and the older generation tests are ridden with high false positive rates.[1,28] The newer generation tests have better accuracy and are recommended for ambiguous cases where drainage of liquor is not clearly visualised.[29]

The decision for timing of delivery in PPROM is a delicate balance that considers risks of prematurity to the neonate brought about by early delivery versus increasing risks of infection from prolonging the pregnancy. Further management is therefore dependent on gestational age and varies according to local protocols. In general, expectant management is carried out until 34 or 37 completed weeks when delivery is initiated. During this period, women are observed for signs of CCA. Once infection is suspected or confirmed, delivery is often recommended as the risk to the mother and neonate increases drastically.[1,30]

Outcomes for neonates born from pregnancies complicated by chorioamnionitis in PPROM are poorer than for those born from PPROM alone.[8,9] Administration of antibiotics significantly improves outcomes and is recommended as soon as the diagnosis is made.[1] In addition, the sooner antibiotics are started after diagnosis or suspicion of chorioamnionitis, the better the outcomes.[31,32] Further, the duration of chorioamnionitis has been shown to correlate with neonatal outcomes. Rouse *et al*[β] demonstrated an increase in proportion of neonates with $\leq$3 score in the 5 minute Apgar and increased neonatal mechanical ventilation in pregnancies with a longer duration of chorioamnionitis before delivery.

Early identification of pregnant women with chorioamnionitis and those whose neonates are at high risk for neonatal sepsis may inform early interventions for delivery and antibiotic use and reduce complications related to infections even before clinical signs and symptoms of infection are evident. In addition to clinical features of chorioamnionitis, markers in the maternal blood,[33–35] amniotic fluid[12,36,37] or vaginal fluid[38–40] have been explored as potential aids to the diagnostic process. Some of these tests may be able to diagnose chorioamnionitis in mothers with PPROM on expectant management before characteristic clinical features appear.

Index Tests

Inflammatory markers are biomarkers whose production is increased in the presence of infection or inflammation. These markers may be increased locally at the site of infection or may be present in the systemic circulation. This allows their levels to be assayed from a peripheral blood sample and results used to assess likelihood of an ongoing infectious or inflammatory process. The ideal biomarker is one that facilitates early rapid diagnosis, predicts course and prognosis of disease and guides therapeutic decisions.[41] Several

biomarkers have been evaluated for prediction of chorioamnionitis in pregnant women. There is no consensus on which biomarker is most useful in the context of PPROM. Despite this, many biomarkers continue to be assayed, often repeatedly in women with PPROM with results influencing clinical decision making.[21]

C-reactive protein(CRP) is an acute phase protein synthesized by the liver in response to tissue injury, infection and inflammatory diseases.[42] CRP levels begin to rise after 12-24 hours and peaks at 48 hours.[42] Its use in diagnosis and monitoring of different inflammatory processes is well documented.[43,44] CRP has been evaluated in the prediction of chorioamnionitis in several studies.[45–48] Its role in PPROM is not well defined and guidelines do not recommend its routine use[1]. Despite this, it continues to be used routinely in many settings for the diagnosis of chorioamnionitis.[21]

A newer marker, Procalcitonin(PCT), is a pro-peptide precursor of calcitonin whose levels rise rapidly in the presence of bacterial infection.[49] PCT under normal circumstances is produced in the thyroid gland. In systemic inflammation, particularly bacterial infection, PCT is produced in large quantities by extra-thyroidal neuroendocrine tissues.[49,50] It is detectable in the circulation within 2 to 4 hours of the insult and peaks within 6 to 24 hours. Further, its levels parallel the severity of the inflammatory insult or infection.[50] PCT can distinguish bacterial infection from non-infectious inflammatory conditions or viral infections and its production is not affected by anti-inflammatory and immunosuppressive states.[49–51] PCT has been used for diagnosis and prognosis of infectious diseases and in guiding antibiotic therapy.[43,52–54] A number of studies have explored the role of PCT in the diagnosis of chorioamnionitis with variable results.[55,56]

Interleukin 6 (IL6) is a cytokine whose levels are elevated in most inflammatory states. It is both pro-inflammatory and anti-inflammatory in action.[57] It is an activator of the immune system and participates in switching from the innate to acquired immunity. IL6 has been found useful in early diagnosis of bacterial infections in specific clinical settings.[58–60] Maternal serum levels have been assessed for PPROM and microbiological invasion of the amniotic cavity.[61] Its levels have also been assessed in amniotic fluid[12,62,63] as well as in umbilical cord blood[64] and findings suggest a useful role in prediction of infection and related outcomes. Elevated levels in foetal plasma are strong indicators of a foetal inflammatory response and predicts severe neonatal morbidity.[65] Because of this it is now considered essential to the

diagnosis of foetal inflammatory response syndrome. Its role when assayed in maternal blood is less clear.[61]

Alternative Tests

There are several other biomarkers that can be assayed in maternal blood to predict infection. The list includes, but is not limited to: White Cell Count[35,66], Erythrocyte Sedimentation Rate,[28,66] Interleukin 1,[34] β HCG,[34] Interleukin 8,[67] Interleukin 33,[68] Tumour Necrosis Factor α,[69] Vascular Endothelial Growth Factor,[70] Granulocyte Colony Stimulating Factor,[69] urokinase plasminogen activator receptor[68] and ST2.[68] Blood cultures have not been found to be beneficial in the diagnosis of chorioamnionitis.[71]

Alternative samples that have been assessed include amniotic fluid obtained via amniocentesis[12,36,37] or via sampling the vaginal pool of fluid.[38–40] Amniotic fluid culture[62] and amniotic fluid inflammatory markers[12,36,37] have been assessed for presence of intra-amniotic infection and/or inflammation. While some of these tests are promising,[12,13,62] the clinical usefulness is limited by the need to perform amniocentesis. The procedure is complex, performed in specialist centres only and has risk of complications.[72,73] It is also not practical to obtain repeat samples in the setting of prolonged expectant care. Cervical or vaginal sampling of fluid may be technically easier but the sample is unsuitable for culture due to presence of bacterial contamination.[12] Cord blood samples can also be used to confirm presence of infection but this sample can only be obtained after delivery. While it may influence the management of the neonate,[17,61] its role in influencing management of PPROM is limited.

Reference Standard

There is no consensus on what would constitute a suitable reference/gold standard for the diagnosis of chorioamnionitis. Several options exist: CCA defined by specific clinical criteria, HCA or funisitis based on objective histological assessment of the placenta or positive amniotic fluid culture of an appropriately collected sample of amniotic fluid. CCA may be present without evidence of HCA[23] and HCA may be present without clinical features of chorioamnionitis.[74] A positive amniotic fluid culture may be present with no evidence of inflammation[75] and inflammation may be present with negative amniotic fluid culture.[13,76]

From a clinical and management perspective, CCA may be considered a suitable reference standard. Presence of clinical features of chorioamnionitis correlates well with poor maternal and neonatal outcomes.[3,6] The diagnosis can also be ascertained in various clinical settings even where resources are limited. A diagnosis of CCA plays great influence on the management of PPROM and guidelines recommend active surveillance for its clinical signs and a change in management once a diagnosis is made.[1] However, false positive rates can be high as the individual features of CCA are non-specific.[1,15] Fever, for example, may occur in normal labour, epidural analgesia or in the presence of other maternal infections such as urinary tract infections. Uterine tenderness may also be found in placental abruption, degenerating fibroids or other non-obstetric conditions. For this reason, we opted not to use CCA as a reference standard for this review.

Some authors have suggested that the true gold standard for intra-amniotic infection is amniotic fluid culture of an appropriately collected sample of amniotic fluid.[15,16] This method is however greatly affected by sample handling and culture methods. Very fastidious organisms may be difficult to culture in routine clinical settings. Where appropriately carried out such as in research settings, amniotic fluid culture may be a reliable reference standard. Results of amniotic fluid culture in these settings have been shown to correlate well with results of histologic studies of the placenta.[62] Amniotic fluid culture correctly detects infection but may exclude cases of inflammation without infection.[77] Some authors suggest that inflammation is a more important predictor of outcome than infection alone. Shim *et al*[76] found intra-amniotic inflammation correlated more with preterm delivery in PPROM than positive amniotic fluid culture. Intra-amniotic inflammation has also been shown to correlate well with adverse perinatal outcomes, infection without inflammation (colonisation) being relatively benign.[75] For these reasons, we opted not to use amniotic fluid culture as a reference standard for this review.

HCA and funisitis may be deemed suitable reference standards. The assessment is objective where standard criteria are used and allow grading for severity.[13,22] Clinical features do not accurately correlate with presence of HCA or funisitis[78] and in some cases, histologic evidence of inflammation is present with no evidence of infection.[79] A complete assessment of the placenta is only possible after delivery and is not routinely carried out in non-specialised centres. In clinical practice, a diagnosis of HCA is more influential for the management of the neonate after birth and less for decision-making during pregnancy. Since HCA and funisitis correlate well with neonatal outcomes[63,80] and because of the

objectivity of assessment, we opted to use HCA and funisitis as the reference standard for this review.

Infectious complications of PPROM have greater impact on the neonate than on the mother. As a result, prediction of or ruling out neonatal sepsis is an important goal of care. Early onset sepsis (EONS) is often due to vertical transmission from contaminated amniotic fluid or during vaginal delivery from bacteria colonizing or infecting the mother's lower genital tract while late onset sepsis is usually acquired from the care giving environment.[81] Because EONS correlates more strongly with chorioamnionitis than late onset neonatal sepsis,[81] we opted to consider EONS as the outcome of interest for this review. The maternal inflammatory markers were assessed for their prognostic/ predictive role.

LITERATURE REVIEW

The role of inflammatory markers in the context of PPROM has been systematically reviewed.[82,83] Trochez-Martinez *et al*[82] looked at the use of CRP in the prediction of HCA in PPROM. This review that included articles up to the year 2006 found marked heterogeneity between studies and as a result, pooled analysis was not carried out. The reviewers concluded that there was no clear evidence to support use of CRP for early diagnosis of chorioamnionitis.

Van de Laar *et al*[83] also looked at CRP in the context of PPROM. Their review included articles up to 2007. In addition to predicting chorioamnionitis, they also looked at prediction of neonatal sepsis. They found CRP not to be a useful predictor for neonatal sepsis. CRP was only moderately predictive of HCA. Due to significant heterogeneity, pooled analysis on clinical chorioamnionitis could not be performed.

While both these reviews did not recommend use of CRP for predicting chorioamnionitis, their conclusions were largely due to the small number of included studies and the significant heterogeneity. In addition, these reviews assessed use of CRP only and did not consider other inflammatory markers. There have been several primary studies evaluating CRP in this role after 2007.[84,85] Several other markers have also been assessed in the diagnosis of chorioamnionitis and prediction of neonatal sepsis.[48,85,86]

A more recent review looked at various inflammatory markers in the prediction of neonatal sepsis. Su *et al* [17] assessed the performance of PCT, CRP, IL-6 and leucocyte count in the prediction of neonatal sepsis and included articles up to March 2013. This review assessed these markers in maternal serum as well as in cord blood but only assessed the outcome of neonatal sepsis. While neonatal sepsis is an important outcome to consider in PPROM, it is also important to consider maternal outcomes such as chorioamnionitis in women. Further, the review assessed maternal markers in general and was not specific to PPROM. This significantly limits the applicability of its findings in the clinical management of PPROM which is known to be a high risk condition for infectious complications. Diagnostic tests are known to perform differently in different clinical settings and with different patient groups.[54]

Characteristics of previous related reviews are summarised in Table 1. As of 2nd June 2015, we found no registered ongoing reviews on maternal inflammatory markers for PPROM on the available online registers on the International Prospective Register of Systematic

Reviews (PROSPERO) http://www.crd.york.ac.uk/prospero/search.asp, Cochrane Library http://onlinelibrary.wiley.com/cochranelibrary/search and the National Institute for Health Research, NIHR Centre for Reviews and Dissemination http://www.crd.york.ac.uk/CRDWeb/.

Table 1. Summary of Previous and Related Reviews

Author / Publication Year	Review type / Last search date / Data Sources	Review question	Number of Included Studies	Findings / Conclusions	Comments on Review methods
Su 2014[17]	Systematic review March 2013 Medline, EMBASE, Cochrane Library No language restrictions	Index tests: CRP, PCT, IL6, WBC in maternal serum (and cord blood) Outcome: EONS Patient Population: Pregnant women and neonatal populations (not specific to PPROM)	CRP in maternal blood – 8 studies IL6 in maternal blood – 5 studies	Only IL6 in maternal blood was found sufficient as a rule in test for EONS High between study heterogeneity	Strengths: 2 independent reviewers, 3rd for consensus Methodological quality assessment- QUADAS Weaknesses: Unreliable methods for assessing heterogeneity, Pooled analysis despite wide range of cut-offs
Van de Laar 2009[83]	Systematic review 2007 Medline, EMBASE, reference lists of primary studies and known reviews No language restrictions	Index test: Maternal CRP Reference Standard: HCA, CCA, Neonatal Sepsis Patient population: PPROM < 36 weeks	5 studies (HCA – 4 studies, CCA- 4 studies, Neonatal sepsis – 0 studies)	No clear evidence to support use of CRP as an accurate diagnostic test of HCA Poor quality of included studies Studies on CCA were very heterogeneous hence unable to construct reliable SROC curve	Strengths: 2 independent reviewers, 3rd reviewer for consensus Appropriate analysis methods
Trochez Martinez 2007[82]	Systematic review 2006 Medline, EMBASE, Cochrane, reference lists of primary studies and other reviews No language restrictions	Index test: Maternal CRP Reference Standard: HCA Patient population: PPROM <37 weeks	8 studies	No pooling of studies due to significant unexplained heterogeneity No clear evidence to support use of CRP for early diagnosis of chorioamnionitis	Strengths: No language restrictions, protocol, methodological quality assessment Weaknesses: 1 reviewer, Unreliable analytic methods for assessment of heterogeneity, included some papers with term PROM and CCA, unreliable methods of constructing SROC curves
Wiwanitkit 2005[87]	Systematic, (Partially systematic) PubMed	Reference Standard: chorioamnionitis (HCA or CCA) Patient population: PROM, Preterm Labour, Amniotic Infection Syndrome, any gestation (Not limited to PPROM)	6 studies, 466 cases	Maternal CRP is not a good tool for the detection of chorioamnionitis	Weaknesses: Not quite systematic, poor and unreported statistical methods
Bek 1990[88]	Narrative review	CRP		Elevated values of CRP indicate infection and rising values seem to show convincing signs of impending infection	Weaknesses: Not systematic
Ohlsson 1990[89]	Systematic 1980 to 1988 Medline	Reference Standard: Chorioamnionitis, Fetal / Neonatal sepsis Patient population: PPROM	23 studies	An ideal test to predict chorioamnionitis or neonatal sepsis was not found	Strengths: Independent review with pre-set criteria

CRP,C reactive protein; PCT, Procalcitonin; IL6, Interleukin 6; WBC, White Blood Cell Count; PPROM, Preterm Premature Rupture of Membranes; EONS, Early Onset Neonatal Sepsis; QUADAS, Quality in Diagnostic Accuracy Studies; HCA, Histologic Chorioamnionitis; CCA, Clinical Chorioamnionitis; SROC, Summary Receiver Operator Characteristics; PROM, Premature Rupture of Membranes.

JUSTIFICATION

The burden of PPROM remains high and the subsequent maternal and neonatal complications are significant. In sub-Saharan Africa, infection related complications continue to contribute significantly to maternal and neonatal morbidity and mortality. Early diagnosis or prediction of infectious complications of PPROM may lead to better outcomes by triggering timely change in the management of these pregnancies.

Inflammatory markers have been found to be beneficial in the diagnosis and prognosis of other infections. CRP and PCT are now in routine use in the management of severe infections in other clinical settings. Chorioamnionitis and PPROM are unique conditions in the unique physiological state of pregnancy and performance of inflammatory markers may also be unique.

Several studies have assessed the predictive and diagnostic role of these markers in PPROM. These studies and existing reviews are not conclusive on which tests to use in the diagnosis and prediction of infectious complications of PPROM. Despite this, several tests and combinations of tests continue to be used in the management of PPROM. Use of these tests adds cost to care and may result in inappropriate management decisions regarding delivery and/or parenteral antibiotic use. The findings of this study will advise on use of tests in PPROM as well as facilitate their interpretation based on current evidence.

Both CRP and PCT are available at the Aga Khan University Hospital, Nairobi. Recommendations for or against their use from the findings of this review will be directly applicable to this centre as well as other centres that have access to these tests.

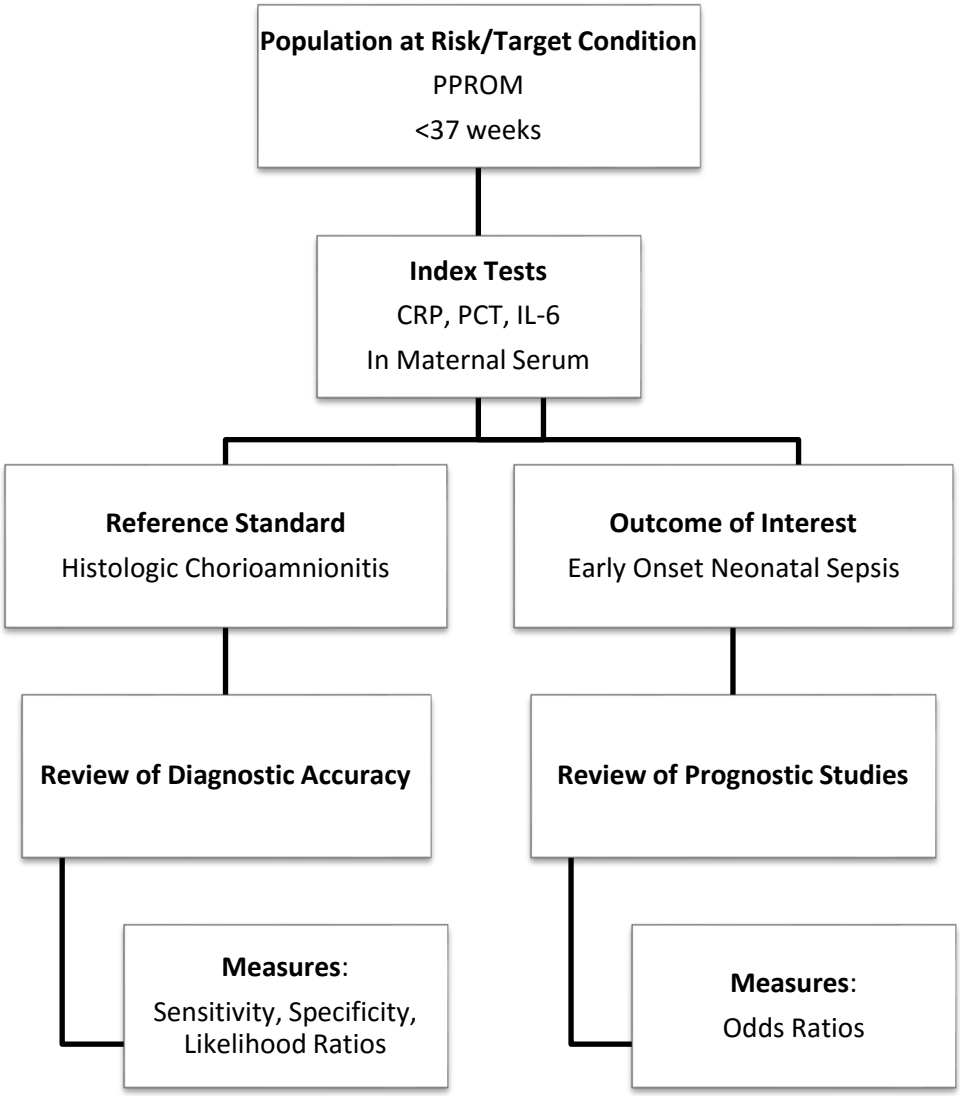
Maternal serum is an easy to obtain sample for laboratory assessment. It is also suitable for repeated assays in the setting of prolonged care. Assessment of inflammatory markers in maternal serum improves applicability of the findings to routine clinical care including care in low resource settings. This is in contrast to tests conducted on amniotic fluid that would only be applicable in specialist centres where amniocentesis is carried out.

REVIEW QUESTION

In pregnant women with PPROM, can maternal serum inflammatory markers be used to diagnose chorioamnionitis or predict early onset neonatal sepsis?

Conceptual Framework

Figure 1. Conceptual framework for the review



PPROM, Preterm Pre-labour Rupture of Membranes; CRP, C reactive protein; PCT, Procalcitonin; IL6, Interleukin 6.

OBJECTIVES

Broad objective

To perform a quantitative review on the accuracy of maternal inflammatory markers in the diagnosis of Histological Chorioamnionitis and/or Funisitis and their role in the prediction of Early Onset Neonatal Sepsis in Preterm Pre-labour Rupture of Membranes.

Specific Objectives

1. Obtain the individual and pooled estimates of sensitivity, specificity and likelihood ratios of maternal serum C Reactive Protein (CRP), Procalcitonin (PCT) and Interleukin 6 (IL6) in the diagnosis of Histological Chorioamnionitis and/or Funisitis.
2. Obtain the individual and pooled Odds Ratio for maternal serum CRP, PCT and IL6 in the prediction of Early Onset Neonatal Sepsis.
3. To assess for sources of heterogeneity in the estimates of diagnostic accuracy and predictive role.

METHODOLOGY

Study Design

The study design was a systematic review. The review had two components: A review of diagnostic accuracy and a review of prognostic studies. The diagnostic accuracy review followed methodological approaches recommended in the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy.[90] The prognostic review followed methods recommended by the Cochrane Prognosis Methods Group.[91]

A protocol was prepared in accordance with the Cochrane recommendations[92] and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline[93] and registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42015023899.[94]

Criteria for considering studies for the review

Population:

We considered studies of pregnant women with PPROM before 37 completed weeks of gestation.

Test(s):

The tests of interest were CRP, PCT and IL6 performed on a maternal blood sample collected prior to delivery. All methods of assay were considered. Studies were included regardless of the cut-off used. Each marker was assessed separately for its diagnostic and predictive role.

Reference Standard

For chorioamnionitis, we considered histologic chorioamnionitis and/or funisitis (HCA/Funisitis) as the reference standard. For this reference standard, a definition or diagnostic criteria needed to have been provided. Alternatively, a specification of histological assessment of the placenta, microscopic assessment of the placenta or assessment of the placenta by a pathologist for HCA/Funisitis was considered sufficient.

For neonatal sepsis, the clinical outcome of interest was EONS. For neonatal sepsis to be considered to be early onset, a specified timeline within 1 week of delivery was accepted.[81,95–97] The designation 'early' was also accepted. Studies were also included if the

methodology specified that the assessment and designation of the outcome was carried out at any time within 1 week of delivery. We included studies where neonatal sepsis/infection was defined by clinical and/or laboratory features. Studies that addressed neonatal sepsis/infection without specifying the time duration when the diagnosis was made were excluded.

Study Designs

We included studies in which the results of the index test used were compared with the reference standard of HCA/funisitis and/or the clinical outcome of EONS. Any of the following study designs were eligible: Clinical trials, prospective cohort studies, retrospective cohort studies, cross-sectional studies and case control studies. The specific designation of a 'diagnostic study' or a 'prognostic study' was not a requirement. Case reports and case series were not eligible.

For the diagnostic accuracy review, included studies had to have data to allow formation of 2X2 tables and calculation of indices of diagnostic accuracy for the reference standard of HCA/funisitis. An outline of the inclusion and exclusion criteria is given in Table 2.

Table 2. Inclusion and Exclusion Criteria

Inclusion criteria	**Exclusion criteria**
Population: Preterm pre-labour rupture of membranes	Case reports/ case series
Index test: CRP, PCT and/or IL6 assayed in maternal serum	Gestation not specified
Reference standard: Histologic Chorioamnionitis and/or Funisitis OR outcome of interest: Early Onset Neonatal Sepsis	
Marker was assessed prior to delivery	
Any method of laboratory assay	
A cut-off is specified, Any cut-off	
Data allowed formation of 2x2 tables for each test separately	

CRP, C reactive protein; PCT, Procalcitonin; IL6, Interleukin 6.

Search Methods for Identification of Studies

We aimed to identify all relevant studies published in peer reviewed journals.

Electronic Searches

We conducted an electronic search on MEDLINE, EMBASE and The Cochrane Library databases. All databases were searched from their inception to the last Search date - 29th October 2015. Search terms for the electronic search were a combination of free text terms and subject headings that referred to the index test and target population only.[98] For the test, search terms included C Reactive Protein, Procalcitonin or Interleukin 6 and their word variants. For the population / target condition, 'Rupture of Membranes' and its word, spelling and phrase variants were included in the search terms. We did not use any filters or search terms for the study design.[99,100] Specific terms of 'diagnostic study' and 'prognostic study' were not included in the search terms. To avoid excluding necessary studies, search terms that specified the gestation were not used. This was to avoid excluding studies that may have included a spectrum of gestational ages but provided data enabling extraction for the preterm gestation subgroup.

There were no restrictions for language, publication dates or geographical setting in the electronic search. Where the database allowed, the limit for 'Humans' was applied.

The specific search strategies for the 3 databases are provided in Appendix 1 (Appendix 1a: Search strategy for MEDLINE, Appendix 1b: Search Strategy for EMBASE and Appendix 1c: Search strategy for The Cochrane Library).

Searching Other Resources

Reference lists of all included studies and previous related reviews were searched manually to identify further relevant studies. It was decided *a priori* that unpublished studies and other supplementary approaches to obtain data would not be pursued as the turnaround time for these would not fit within the time frame for the dissertation.

Study Selection

Study selection was done in two stages: All selected articles from the various sources were pooled together into the reference management software, Endnote X7. We also used Microsoft Excel 2010 workbook templates from the University of Texas School of Public Health Library.[101] Duplicates were removed initially by the automated 'search for duplicates'

Endnote feature. Further duplicates were removed by matching author names, study titles and article page numbers in the Microsoft Excel Workbooks.

Titles and/or abstracts of the articles were screened independently by 2 reviewers. Disagreements were resolved by consensus with planned resolution of conflicts by a third reviewer. Reviewers were blinded to author names and year of publication during the screening stage. Despite no language restrictions in the electronic search stage, non-English articles were excluded from further steps in the review due to time and resource constraints that limited the ability to correctly translate non-English articles.

English articles that appeared to meet the inclusion criteria or that had insufficient information in the title or abstract to make the decision for inclusion proceeded to the next step. In the second step, full texts of selected articles were obtained. These were reviewed in depth and included in the review if eligibility criteria were met. Reviewing of full texts was done independently by 2 reviewers. Disagreements were resolved by consensus with planned resolution of conflict by a third reviewer. Reviewers were not blinded in the full text review. Reasons for excluding articles at full text review were outlined for each excluded study. Inter-rater reliability was assessed by calculating percentage agreement and Cohen's kappa for both the screening of titles and abstracts and for the reviewing of full texts stages.

Data Extraction

We designed a data extraction form and piloted it on 3 randomly selected included studies. The form was then modified and improved for clarity and to include omitted items. The final version of the data extraction form is included in Appendix 2. Extracted fields included: study design, setting, inclusion criteria, gestational age range, index test, method of assay, cut-off used, timing of index test in relation to delivery and prior antibiotic use. We also extracted components of the 2x2 table and/or indices of diagnostic accuracy such as Sensitivity, Specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV). Data extraction was done independently by two reviewers and disagreements resolved by consensus.

Where a study reported data on a wide range of clinical diagnoses or where the study reported on ROM over a wide range of gestational age, the study was included only if it was possible to extract data for the subgroup with PPROM or for the preterm (<37 weeks)

subgroup. Where it was not possible to extract data from a study that otherwise met inclusion criteria, authors were contacted by email and requested to provide 2x2 table data for their specific studies. Authors were also contacted for conflicting or unclear data.

Study Methodological Quality Assessment/ Risk of Bias in Individual Studies

For the diagnostic accuracy review, the Quality Assessment of Diagnostic Accuracy Studies 2, (QUADAS-2)[102] tool was used to assess the methodological quality of included studies and to provide judgement on their risk of bias and applicability of findings to the review question. A review specific quality checklist derived from the QUADAS-2 tool was designed and is provided in Appendix 3.

For the prognostic review, the Quality in Prognostic Studies (QUIPS)[103] tool was used to assess methodological quality of included studies with regards to risk of bias. A review specific quality assessment tool derived from the QUIPS tool was designed and is provided in Appendix 4.

For each tool, two reviewers independently scored the included studies. Disagreements were resolved by consensus with planned resolution of conflict by a third reviewer. Graphical representations of individual study judgements and summary judgements of included studies were prepared. The judgements of selected domains in the quality assessment were used to categorize studies for investigation of heterogeneity.

Assessment of Publication Bias across Studies

No assessment of publication bias was performed for the diagnostic review as included studies were few or too heterogeneous.[92,104] For the prognostic review, assessment of publication bias was not performed due to the small number of included studies.[105,106]

Data Analysis

Description of Included Studies

A flow diagram was produced to display the study selection process.[93] A detailed descriptive analysis of the included studies was carried out and summary tables prepared. Characteristics described in the studies included: study design, study setting, gestational age range, characteristics of index test, diagnostic criteria of reference standard/outcome of interest and diagnosis and management of PPROM.

Synthesis/Analysis of Results

The analyses were conducted using Cochrane Review Manager (RevMan) version 5.3 (Copenhagen), Stata™ 12.1(College Station, Texas) and SAS® University Edition 2016 (Cary, North Carolina).

For the diagnostic accuracy review, we extracted and tabulated True Positive, True Negative, False Positive and False Negative values for each test in each study against the reference standard. Where 2x2 tables were not directly provided, we calculated components of the 2x2 table from other diagnostic indices provided and prevalence of the outcome in the included studies. The calculator function in Cochrane Review Manager 5.3 was used for this. Individual estimates of Sensitivity, Specificity and Likelihood Ratios (LRs) were calculated and tabulated. Meta-analysis was carried out if the number of studies in each category was ≥3.

Forest plots were constructed to display each study's Sensitivity, Specificity and corresponding 95% confidence intervals (CI). Summary Receiver Operator Characteristic (SROC) curves were constructed for each test using the Rutter and Gatsonis' Hierarchical SROC (HSROC) model.[107] We obtained model parameters in Stata and inputted these into RevMan for construction of the curves.[108] This method is a random effects model and it accounts for the correlation between sensitivity and specificity across the studies with changes in threshold.[107,108] It also makes the most use of the data as studies are pooled regardless of differences in cut-offs.[109] Where studies used the same cut-off we used the HSROC model to obtain Summary Sensitivity and Specificity and corresponding 95% CI for that cut-off. We then pooled all studies regardless of cut-off and constructed an SROC plot to demonstrate the changes in specificity and sensitivity with the different cut-offs. For this analysis, data for 1 cut-off per study was used.

For the prognostic review, odds ratios (ORs) were calculated from the 2x2 tables and presented in forest plots. Meta-analysis was carried out if the number of studies in each category and with the same cut-off was ≥3. Pooled unadjusted ORs and corresponding 95% CIs were calculated and presented on forest plots. Random effects models were used.[110] Odds Ratios were preferred over hazard ratios because the time duration for the outcome of EONS is already specified in the definition. Odds Ratios are also more likely to be obtained from different study designs and would make more data available for the analysis.

Exploration of Heterogeneity between studies

For the diagnostic accuracy review, heterogeneity was initially assessed by visual inspection of forest plots and 95% prediction regions on SROC curves.[108] Further exploration for causes of heterogeneity was carried out where the number of studies exceeded 5 and each subgroup had at least 2 studies. Investigations for heterogeneity evaluated the following as possible sources: assay type and characteristics from the QUADAS-2 quality assessment, specifically, risk of bias in the patient selection domain, nature of cut-off (pre-specified or not) and interval between sampling and delivery. Subgroups were created according to the above characteristics and separate SROC curves were constructed for each subgroup using the NLMIXED procedure in SAS. The binary characteristics were added as covariates to the model and parameters obtained inputted into RevMan. For simplicity, the shape parameter was assumed to be the same in all the curves. Chi squared test was used to compare the -2 Log likelihoods to test for differences in SROC curves between subgroups. Covariates were applied to the model one at time and curves compared for each characteristic in turn. We did not construct models with more than one covariate due to limited power in the setting of few studies.[109,111]

For the prognostic review, statistical heterogeneity was assessed by using the Chi squared test for heterogeneity and using I^2 to assess inconsistency across studies. Subgroup analysis was not conducted due to the small number of included studies.

Sensitivity Analysis

Sensitivity analysis was only performed for CRP in the diagnostic review as the other categories had insufficient numbers of studies. We investigated whether using a narrower gestational age for inclusion to the review or limiting the review by year of publication would change the findings of the review. We also evaluated whether limiting the review to studies that had low concerns for applicability would alter the review findings. Pairs of SROC plots were constructed, one with all included studies and the other with fewer studies limited by the characteristic under evaluation. Comparison of the plots was done visually.[109]

Ethical Consideration

The review did not involve any intervention or collection of primary data. Scientific review of the proposal was conducted by the Aga Khan University (AKU) Research Committee after which formal exemption from ethical review was obtained from the AKU, Nairobi Health

Research Ethics Committee. Written waiver of ethical review (2015/REC -33) is provided in Appendix 5.

RESULTS

Study Identification

The electronic database search identified 2126 records. Of these, 732 were duplicates (62 internal duplicates, 670 external duplicates) leaving 1394 unique records for screening (see Table 3). Titles and abstracts of these 1394 records were screened and 1274 excluded. The remaining 120 articles were eligible for full text review.

Table 3. Summary of Results of Electronic Search*

Data Source			**Limits**			**Duplicates Results**			
Database	Interface	Last Date searched	Language limits	Date range	Other Limits	Items found	Internal duplicates	External duplicates	New Items
Medline®	Ovid	29/10/2015	None	1946 to October, week 4 2015	Human	885	28	0	857
Embase®	Ovid	29/10/2015	None	1947 to 28 October 2015	Human	1177	33	643	501
Cochrane Library	Wiley	29/10/2015	None	-	Human	64	1	27	36
					Totals	2126	62	670	1394

*Table modified from Vonville.[101]

One additional article[112] was identified by searching reference lists of included articles and previous related systematic reviews. Thirty six of these were published in languages other than English and were therefore excluded from further review. We were unable to obtain 3 full texts despite extensive search through a network of libraries. Eighty one articles were taken through full text review. Of the reviewed full texts, 42 articles were deemed eligible.

We were unable to extract or derive components of the 2x2 table for 20 of these articles. In some of these articles 2x2 data was provided but was not limited to the specific patient group with PPROM. Three articles had 2x2 data that was conflicting or unclear. Authors of these 23 articles were contacted via email and requested to provide the 2x2 data. A 10 week period was allowed for author feedback. Authors of 1 article[113] responded but were

unable to provide the data. As a result all 23 articles with missing or conflicting 2x2 data were excluded.

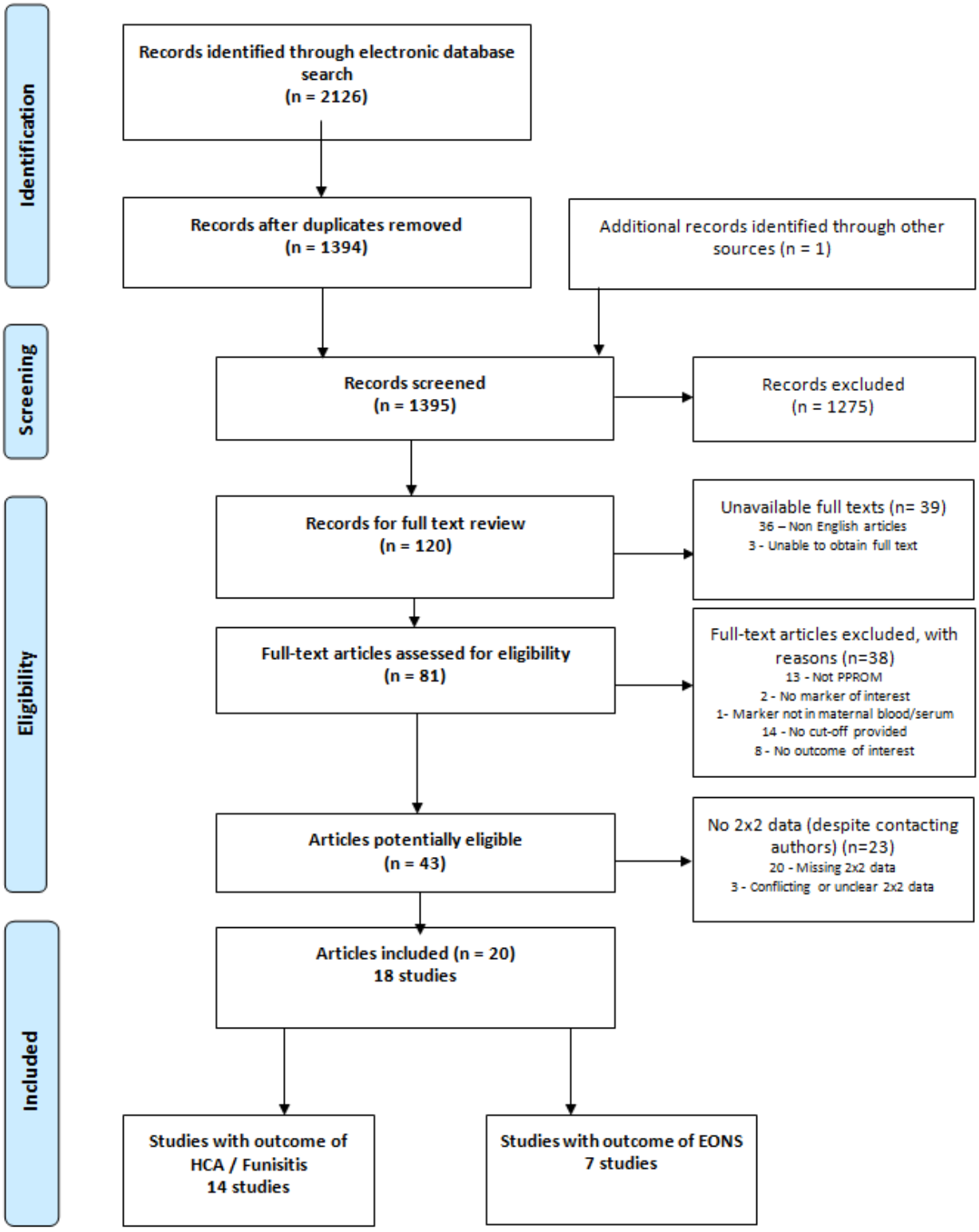
Eighteen studies (from 20 articles) met the inclusion criteria and were included in the final review. The results of the search, screening and selection of studies are summarised in Figure 2.

Of the 18 included studies, 14 studies assessed HCA and/or funisitis as the reference standard and 7 studies assessed EONS as the outcome of interest. Of these, 3 studies[56,114,115] assessed both outcomes.

Inter-Rater Reliability between Reviewers

Of the 1395 articles screened, the screeners agreed on outcomes of 1339 articles, 96% percent agreement, Cohen's kappa 0.75. All disagreements were resolved by consensus between the two reviewers. Of the 81 full texts that were reviewed, reviewers agreed on 79 articles, 98% percent agreement, Cohen's kappa 0.95. All disagreements were resolved by consensus.

Figure 2. Study Flow Diagram



PPROM, Preterm Pre-labour Rupture of Membranes. HCA – Histologic Chorioamnionitis. EONS – Early Onset Neonatal Sepsis. Figure modified from the PRISMA statement[93].

The results of the review are presented under 3 broad areas:

A. The diagnostic review – Inflammatory markers in maternal serum for diagnosis of HCA and/or Funisitis
B. The prognostic review – Inflammatory markers in maternal serum and their role in prediction of EONS
C. The summary of findings[116]

Inflammatory markers for diagnosis of Histologic Chorioamnionitis and/or Funisitis

Characteristics of Included studies

Characteristics of the 14 included studies that assessed HCA and/or funisitis (HCA/Funisitis) are summarised in Table 4. These studies were published between 1983 and 2014 and were conducted in 8 countries. All studies were conducted in hospital inpatient settings with majority at teaching/university hospitals. In total, 761 women were included with 361 episodes of HCA/funisitis reported. Prevalence of HCA/funisitis ranged from 21% to 63% (Median 41%, Inter-Quartile Range (IQR) 36% to 53%). Majority of the included studies were prospective cohort design, with only 1 study[117] being retrospective cohort design.

Characteristics of participants

All studies had no restrictions on maternal age or parity. All included studies were of preterm gestation (<37 weeks) at the time of PROM. The gestational age range for eligibility of participants varied greatly among the included studies (see Table 4).

However, the actual gestational age range for the participants who were included into the study was reported in only 9 studies.[56,114,115,118–124] The methods used to assess gestational age were unreported in most studies[114,115,117–119,121,125–127] with only 3 studies[56,124,128] reporting that they used a combination of last menstrual period and ultrasound.

Table 4. Characteristics of Included Studies, Index test against the reference standard of Histologic Chorioamnionitis and/or Funisitis.

Study	Year of End of Study	Country	Study Design	No of Participants (excluded)*	Gestational Age (GA) Range Criteria (weeks)	Actual GA at admission or at ROM (weeks)	GA at delivery (weeks)	Time from ROM to delivery	Antibiotics	Steroids	Tocolytics	Reference Standard	Prevalence of outcome (%)
Farb 1983[125]	1981	Minnesota, USA	Prospective Cohort	31(7)	20 to 36	NR	NR	NR	NR	Yes	Yes	HCA and Funisitis	5/24(21)
Hawrylyshyn 1983[128]	1982	Canada	Prospective Cohort	54(2)	20 to 34	NR	NR	NR	None	Yes	Selective	HCA	26/52(50)
Ismail 1985[118]	1982	Chicago, USA	Prospective Cohort	100(0)	26 to 35	Mean 31 Range 26-37	NR	Mean 150 hours SEM 21.7hours, Range 5-1053hours, Median 72hours	NR	No	No	HCA	63/100(63)
Fisk 1987[126]	1986	Saudi Arabia	Prospective Cohort	55(4)	26 to 36	NR	NR	NR	NR	Selective, <34weeks	Selective, <32weeks	HCA	30/51(59)
Yoon 1996[119]	1995		Prospective Cohort	91(28)	20 to 37	Range 20 - 36.7	Range 23.3-41.4	NR	NR	NR	NR	HCA and Funisitis	35/63(56)
Danielian 1991[127]	NR	NR	Prospective Cohort	17(6)	26- ? (preterm)	NR	NR	NR	NR	NR	NR	HCA	4/11(36)
Torbe 2007[56]	NR	?Poland	Prospective Cohort	48(0)	24 to 34	Mean 30.8 SD 3.3 (at ROM)	Mean 31.4 SD 3.0	Mean 5.5 days, SD 8.1 days	Yes	Yes	None	HCA	14/48(29)
Murtha 2007[120]	2004	North Carolina, USA	Prospective cohort	122(15)	22 to 34	Mean 28	Mean 30.0	NR	Yes (all)	Selective (23 to 34 weeks)	NR	Funisitis	54/107(50)

*Number given is the total number recruited, 'excluded' refers to participants whose index test or reference standard data was unavailable or not reported; GA, Gestational Age; USA, United States of America; NR, Not Reported; HCA, Histologic Chorioamnionitis; SD, Standard Deviation; SEM, Standard Error of the Mean.

Table 4 (continued). Characteristics of Included Studies, Index test against the reference standard of Histologic Chorioamnionitis /or Funisitis.

Study	Year of End of Study	Country	Study Design	No of Participants (excluded)*	Gestational Age (GA) Range Criteria (weeks)	Actual GA at admission or at ROM (weeks)	GA at delivery (weeks)	Time from ROM to delivery	Antibiotics	Steroids	Tocolytics	Reference Standard	Prevalence of outcome (%)
Smith 2012[117]	2008	Pennsylvania, USA	Retrospective cohort	73(0)	20-37	NR	Mean 31.0 SD 4.0	Median 4 IQR 1-10	Selective	Selective	NR	HCA	26/73(36)
Perrone 2012[121]	2007	Italy	Prospective Cohort	66(0)	24 to 33	Mean 28.6 SD 4.4 (at ROM)	Mean 30.8 SD 4.1	Mean 16 days, SD 12days	Yes	Yes	Yes	Funisitis	24/66(36)
Gulati 2012[114,122]	2009	India	Prospective Cohort	45(0)	24 to 34	Mean 30.53 SD 2.128	NR	NR	Yes	Yes	NR	HCA	22/45(49)
Canzoneri 2012[123]	2004	North Carolina, USA	Prospective cohort	39(0)	22 to 34	Mean 27.2	Mean 30.0 weeks	NR	Yes (all)	Selective	No	Funisitis	21/39(54)
Oludag 2014[115]	2008	Turkey	Prospective Cohort	32(0)	24 to 34	Mean 28.1 SD 3.3	NR	NR	Yes	Yes	NR	HCA	13/32(41)
Aksakal 2014[124]	2011	Turkey	Prospective Cohort	50(0)	24 to 37	Mean 33.4 SD 2.7	Mean 33.6+-2.4	NR	All	Selective, <34weeks	None	HCA	24/50(48)
Totals				823(62)									361/761 (47)

*Number given is the total number recruited, 'excluded' refers to participants whose index test or reference standard data was unavailable or not reported; GA, Gestational Age; USA, United States of America; NR, Not Reported; HCA, Histologic Chorioamnionitis; SD, Standard Deviation; SEM, Standard Error of the Mean.

Diagnosis of Pre-labour Rupture of Membranes (PROM)

In majority of the studies, diagnosis of PROM made by clinical assessment based on observation of leakage of amniotic fluid from the cervix or pooling of amniotic fluid in the fornix on speculum examination at the time of admission. In some studies, selected cases of suspected PROM underwent further confirmatory testing. A variety of confirmatory tests were used; Amnisure®,[124] Nitrazine test,[118–120,123,125,128] fern test[119,120,123,125] and Actim PROM test®.[121] Three studies did not perform confirmatory testing[114,115,126] and 3 did not report how the diagnosis of PROM was made.[56,117,127].

Management of PPROM

The management of PPROM was largely expectant with monitoring of fetal well-being, surveillance for clinical features of chorioamnionitis (clinical chorioamnionitis) and monitoring for signs of labour. Details of the management were not reported in most studies. Use of antibiotics, steroids and/or tocolytics was incompletely reported in many studies. Where reported, the use was universal or selective dependent on gestational age or clinical features (Table 4).

The reasons for delivery, where reported, included gestational age>34 weeks,[114,124] failed tocolysis or refractory labour,[118,121,125,128] completion of steroids or confirmed pulmonary maturity,[125,128] foetal distress /abnormal cardiotocogram[114,121,125,128] suspected abruptio[121] and/or other obstetric complications that are indications for delivery.[114,124] Four studies specified that clinical features of chorioamnionitis were an indication for delivery.[114,118,125,126] Six studies did not report the reasons for delivery.[56,117,119,120,123,127]

Reference Standard

The reference standards for the review were HCA and funisitis. Most studies assessed HCA as the reference standard, 3 assessed funisitis alone and 2 studies assessed both HCA and funisitis. HCA and/or funisitis was a pathological diagnosis in all studies with most studies specifying a definition/criteria for the standard along with a standard reference.

Index tests

Three index tests were evaluated, CRP, PCT and IL6. Details of the assays are given in Table 5 along with the limit of detection (analytical sensitivity), cut-off (threshold) used and whether this cut-off was pre-specified or determined from the study data.

Table 5. Characteristics of Index Tests for all included studies.

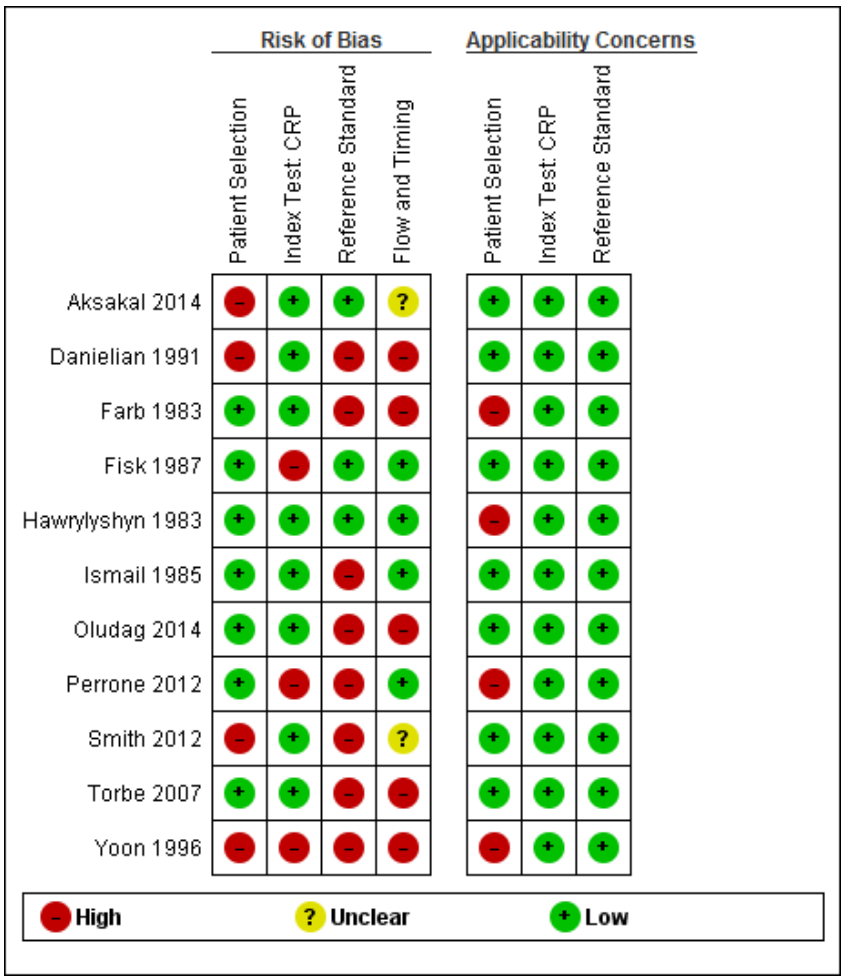
Study Id	Test	Assay Type	Equipment and Manufacturer	Detection limit	Cut off	Predetermined cut off?
Farb 1983[125]	CRP	Nephelometric Immunochemistry	Beckman Instruments Inc., Fullerton, California	1.8mg/L	20mg/L	Yes
Hawrylyshyn 1983[128]	CRP	Rate nephelometry	Beckman Immunochemistry analyser, Beckman Instruments Inc., Fullerton, California	6mg/L	12.5mg/L	Yes
Ismail 1985[118]	CRP	Rate nephelometry	Beckman Immunochemistry analyser, Beckman Instruments Inc., Fullerton, California		20mg/L	Yes
Fisk 1987[126]	CRP	Rate nephelometry	Beckman Instruments Inc., Fullerton, California	6mg/L	20, 30, 35, 40mg/L	No
Danielian 1991[127]	CRP	Rate nephelometry	Beckman Instruments Array Protein System		20mg/L	Yes
Yoon 1996[119]	CRP	Antibody adsorption-particle agglutination assay(Seiken, Japan)	Hitachi 7470 Autoanalyzer, Hitachi, Japan	1mg/L	7mg/L	No
Kayem 2005[129]	CRP	NR	NR	NR	5, 20mg/L	Yes
Torbe 2007[56]	CRP	Immuno-turbidimetry	Olympus AU 560, Olympus Diagnostica, Hamburg, Germany		10mg/L	Yes
Torbe 2010[40]	CRP	Quantitative immune-turbidimetry	Olympus AU 560 System ,Olympus Diagnostica, Hamburg, Germany	NR	10, 15mg/L	Yes
Torbe 2011[130]	CRP	Quantitative immune-turbidimetry	Olympus AU 560 System , Olympus Diagnostica, Hamburg, Germany	NR	10mg/L	Yes
Perrone 2012[121]	CRP	Micro particle Enhanced Turbidimetric Immunoassay	Roche Diagnostic, Manheim, Germany	NR	12, 20mg/L	No
Smith 2012[117]	CRP	NR	NR	NR	50mg/L	Yes
Aksakal 2014[124]	CRP	NR	NR	NR	60mg/L	Yes
Oludag 2014[115]	CRP	Immuno-turbidimetry	Abbott Diagnostics Architect c 16000 system, Abbott Diagnostics	NR	10mg/L	Yes
Torbe 2007[56]	PCT	Immunoluminometric assay	LUMI test, PCT kit, Brahms Diagnostica, Berlin Germany and Luminometer LIA-MAT system 300, CBYK – Sangtec Diagnostic, Dietenbach, Germany	0.1ng/mL	1.9ng/mL	No
Oludag 2014[115]	PCT	Ultrasensitive immunoassay using TRACE(Time Resolved Amplified Cryptate Emission Technology)	Kryptor, Brahms	0.019ng/mL	0.054ng/mL	No
Hatzidaki 2005[61]	IL6	ELISA	Cytoscreen (Biosource Int., Camarillo, California, USA), Bio-tech SERES 900C instrument (Winooski, Vermont, USA).	0.2pg/mL	81pg/mL	No
Murtha 2007[120]	IL6	Ultrasensitive ELISA	Cytokine Core lab, Baltimore, Maryland	1.2pg/mL	1.8, 8pg/mL	No
Gulati 2012[114]	IL6	Standard ELISA, solid phase sandwich ELISA	Diaclone IL6 ELISA kit, Besancon, France	2pg/mL	8pg/mL	Yes
Canzoneri 2012[123]	IL6	Ultrasensitive ELISA	Cytokine Core lab, Baltimore, Maryland	1.2pg/mL	1.98, 5.12, 10.44pg/mL	No

CRP,C reactive Protein; NR, Not Reported; PCT, Procalcitonin, IL6, Interleukin 6; ELISA, Enzyme Linked Immunosorbent Assay.

Methodological Quality of Included Studies

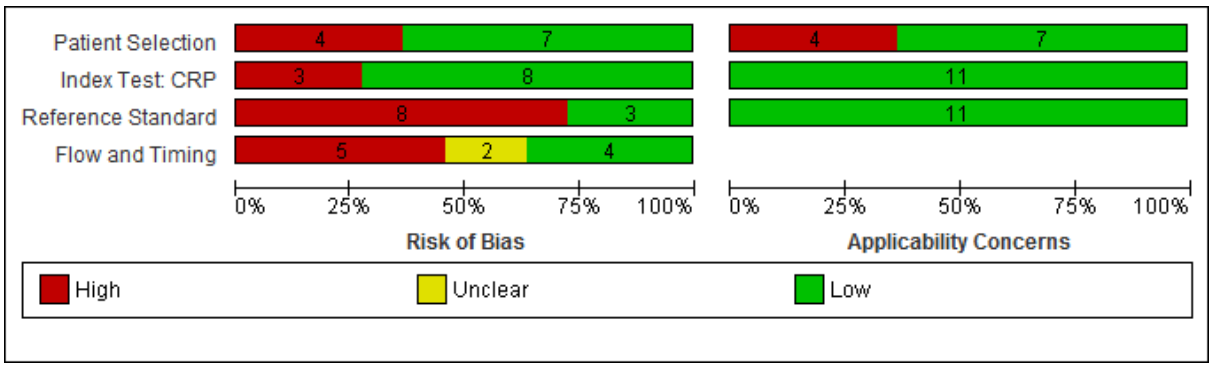
We used the QUADAS-2[102] tool for assessing the quality of included studies. Figures 3 to 5 show the risk of bias and applicability concerns for each included study and the methodological quality summary for the included studies grouped by index test: Figure 3 for studies assessing CRP, Figure 4 for PCT and Figure 5 for IL6.

Figure 3a. Individual Study Risk of Bias and Applicability Concerns for Studies Evaluating C-Reactive Protein for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis.



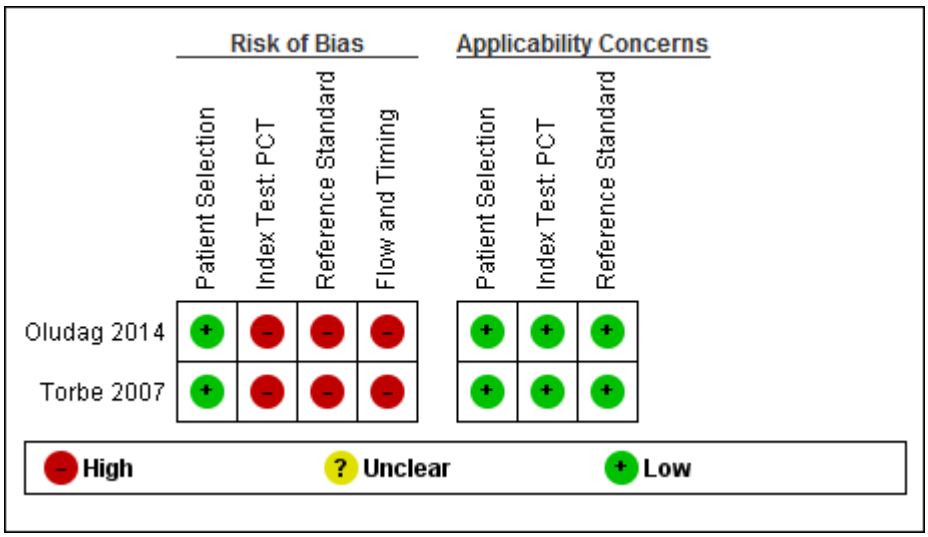
CRP, C-Reactive Protein

Figure 3b. Methodological Quality Summary for Studies Evaluating C-Reactive Protein for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis.



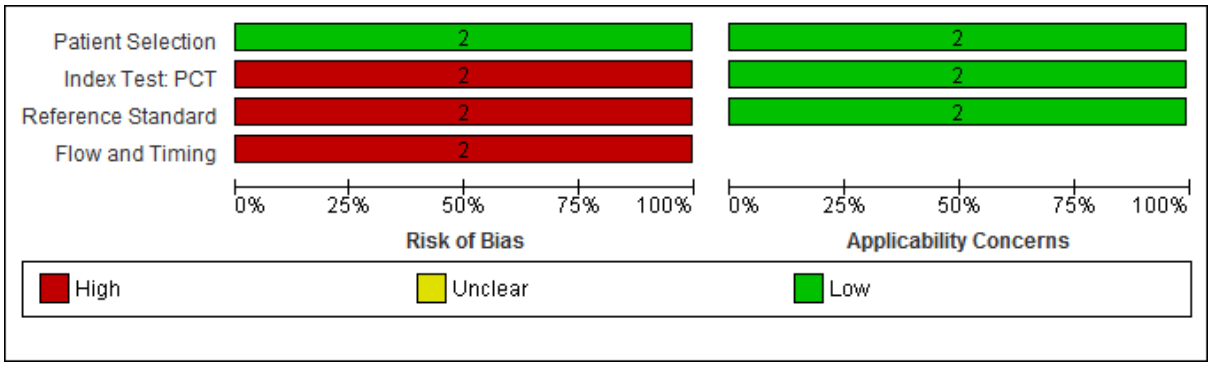
CRP, C-Reactive Protein

Figure 4a. Individual Study Risk of Bias and Applicability Concerns for Studies Evaluating Procalcitonin for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis.



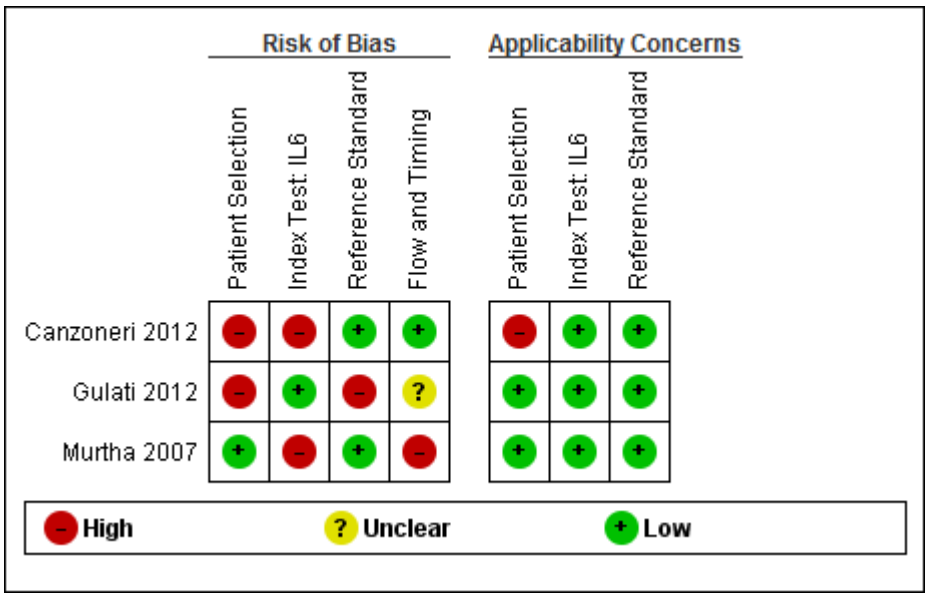
PCT, Procalcitonin.

Figure 4b. Methodological Quality Summary for Studies Evaluating Procalcitonin for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis.



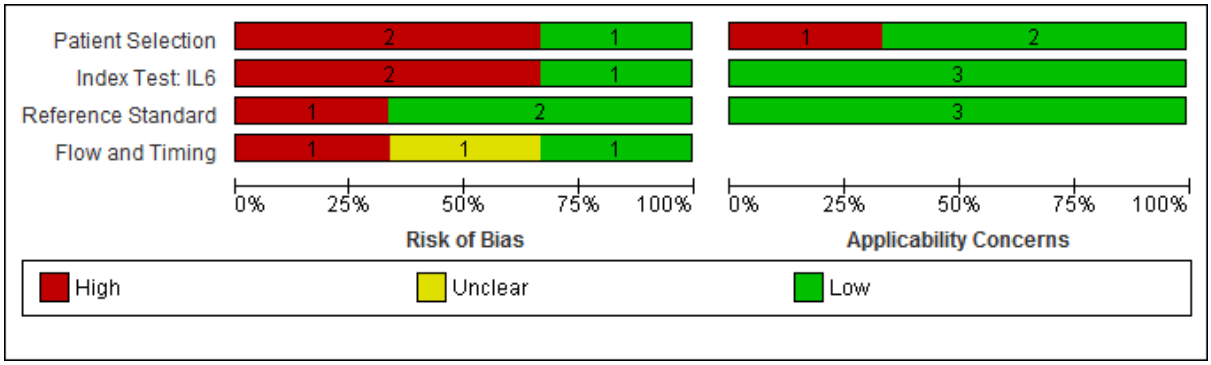
PCT, Procalcitonin

Figure 5a. Individual Study Risk of Bias and Applicability Concerns for Studies Evaluating IL6 for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis.



IL6 – Interleukin 6

Figure 5b. Methodological Quality Summary for Studies Evaluating IL6 for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis.



IL6 – Interleukin 6

Risk of bias in Included Studies

We judged 13 of the 14 included studies to be at high risk of bias in at least one of the four domains. In the 'Patient Selection' domain, we judged 11 of the 14 studies to be at high risk of bias. None of these studies employed a case control design. The method of sampling patients was poorly reported and rated 'unclear' in 10 of 16 studies. Most studies appeared to have used consecutive sampling but did not explicitly state this. We therefore did not factor the sampling method in the judgement of risk of bias for this domain. The risk of bias judgement was largely affected by whether or not the study had inappropriate exclusions. Factors that contributed to inappropriate exclusions were:

a. Limiting the study population to a group of women selected based on their duration after PPROM.[121,123]
b. Failure to explicitly exclude women with clinical features of chorioamnionitis at the time of PPROM or the time of admission.[117,123–125,128,131]
c. Excluding women based on factors related to availability or ability to perform other tests.[119,124]
d. Excluding women based on availability of data.[117,121]
e. Excluding women with common conditions and complications of pregnancy that often coexist with PPROM.[56,114,115,124]

In the 'Index Test' domain, we judged 7 out of 17 index tests to be at high risk of bias. With reference to blinding all studies/tests were considered to be 'blinded' because in all cases the maternal blood sample was collected before delivery and all assays were automated and deemed to be objective. Sources of bias in this domain therefore arose from the selection of a threshold/ cut-off for analysis. In 11 out of 18 tests, the threshold was pre-specified. Several studies[56,115,120,121,123,126,131] selected a threshold from the data after analysis say by selecting optimum sensitivity and specificity from ROC curves.

In the 'Reference Standard' domain, 10 out of 15 studies were deemed to be at high risk of bias related to assessment of HCA and/or funisitis. Reporting of blinding in the assessment of placenta was poorly done in several studies and these were rated 'Unclear' with only 5 studies explicitly reporting blinding.[120,123,124,126,128] Two studies[115,117] used definitions for HCA that were not detailed enough to be deemed objective.

In the 'Flow and Timing' domain, only 5 studies[118,121,123,126,128] were deemed to be at low risk of bias. All studies used the same reference standard for assessing HCA/funisitis in all the included patients. Nine studies[56,114,115,118,121,123,124,126,128] reported data for at least 90% of the

women. There were marked differences in the studies with regard to the time of sampling of the maternal blood relative to delivery. For many studies, it was not specified which sample was used for comparisons with the outcome[114,117,124,125] making it difficult to assess the risk of bias due to time elapsed between maternal blood sampling and delivery, a proxy for the time of placental assessment. Some studies used the sample closest to the time of admission or to the time of PPROM.[56,115] Many studies did not specify the range or average duration of latency after PPROM. For those that reported on latency, the duration between PPROM and delivery was very variable and could last up to several weeks. A maternal sample drawn within at least 72 hours to delivery was deemed appropriate as it was felt the relationship between the result of the index test and the outcome of placental assessment after delivery would be preserved.[102] Only 7 studies[118–121,123,126–128] had samples drawn within this interval.

Applicability Concerns

All included studies had low concerns for applicability with regard to the index test and reference standard as all assessed the index test in maternal blood and before delivery and used HCA or funisitis as the reference standard. There were however some concerns in the 'Patient Selection' domain. Nine studies[117,119,121,125–128]were judged to have high concerns for applicability as they did not explicitly report exclusion of contractions or advanced cervical dilatation (preterm labour).

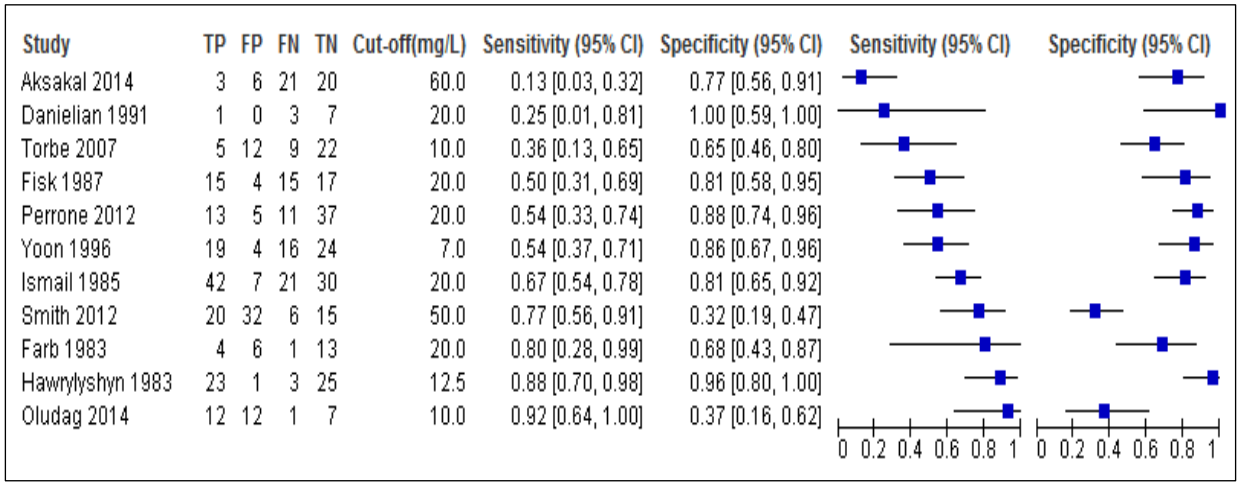
Findings

Studies Evaluating C-Reactive Protein in the Diagnosis of Histologic Chorioamnionitis and/or Funisitis

There were 11 included studies in this category. Sensitivity ranged from 13% to 92% and specificity ranged from 32% to 100%. The range of specificity and sensitivity in these studies is shown in Figure 6a and b.

Several cut-offs (thresh-holds) for CRP were analysed in these studies. The commonest was 20mg/L reported in 5 studies. Two studies reported data at more than one threshold, Fisk *et al*[126] reported at 20mg/L, 30mg/L, 35mg/L and 40mg/L and Perrone *et al* [121] reported at 12mg/L and 20mg/L. For studies that reported multiple thresholds, the threshold of 20mg/L or that closest to 20mg/L was selected for further analysis.

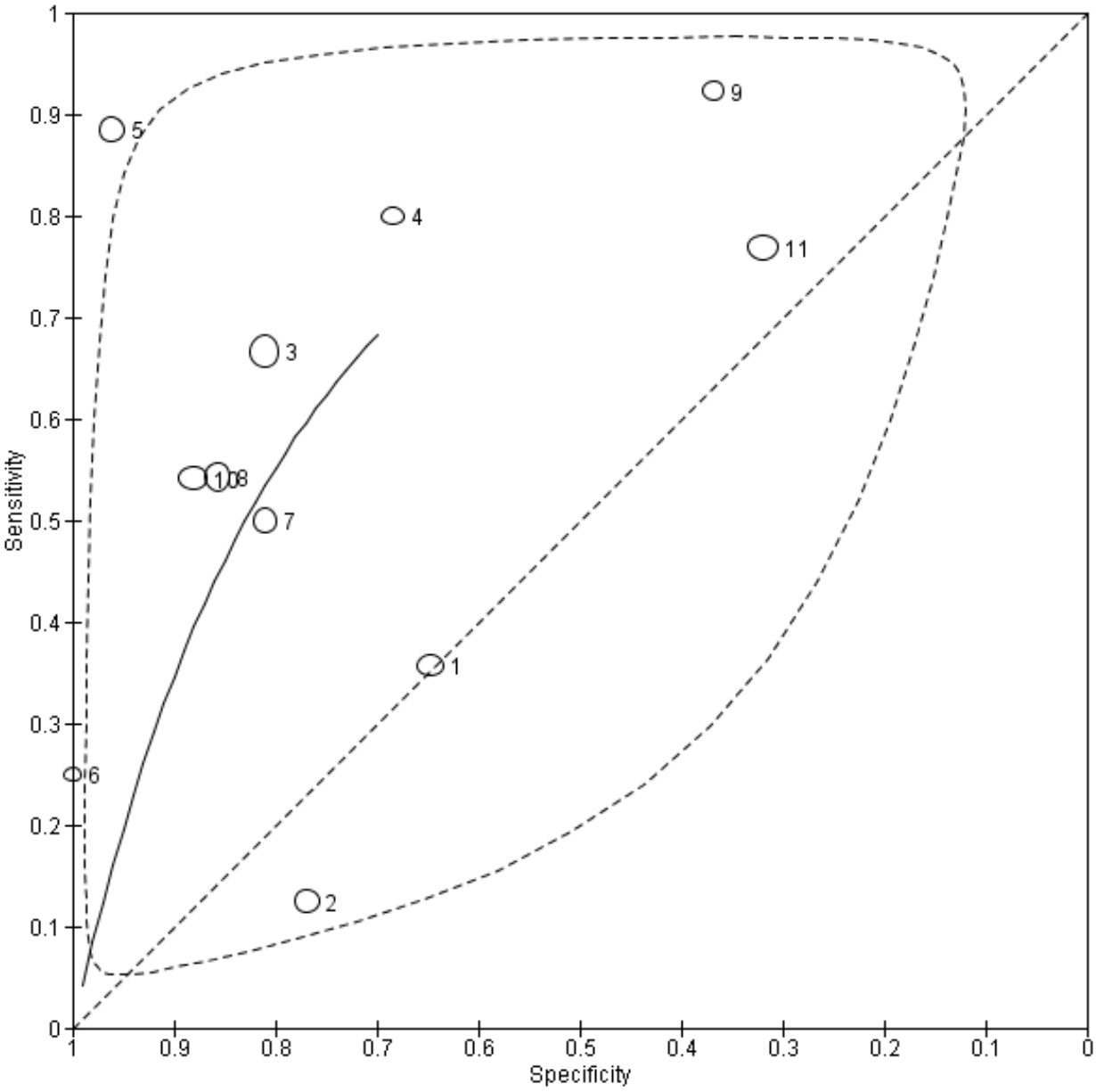
Figure 6a. Sensitivity and Specificity for studies evaluating C- Reactive Protein for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis at all cut-offs.



TP- True Positive, FP- False Positive, FN- False Negative, TN- True Negative, CI- Confidence Interval.

Studies are ordered by Sensitivity in ascending order

Figure 6b. Sensitivity and Specificity for studies evaluating C-Reactive Protein for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis plotted in ROC space.

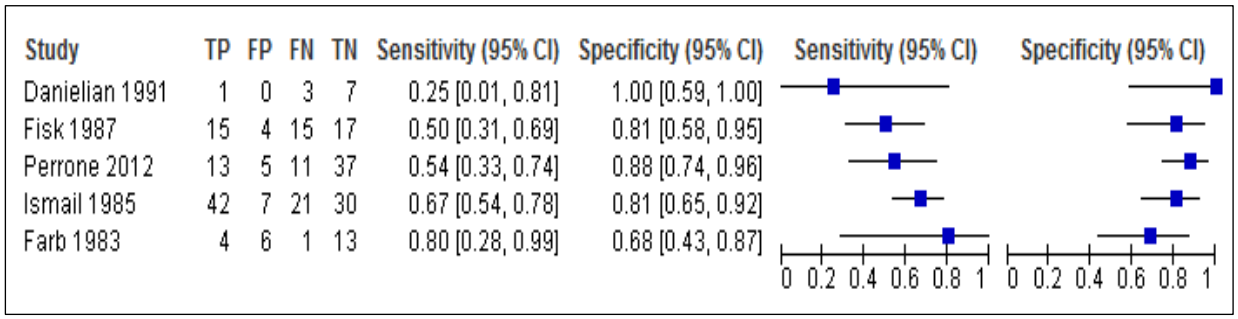


- 95% prediction region.

For further analysis, we selected the studies that used a common cut-off for CRP values. This cut-off, 20mg/L, was used in 5 studies.[118,121,125–127] For these studies, sensitivity ranged from 25% to 80% and specificity ranged from 68% to 100% (see Figure 7a). The pooled

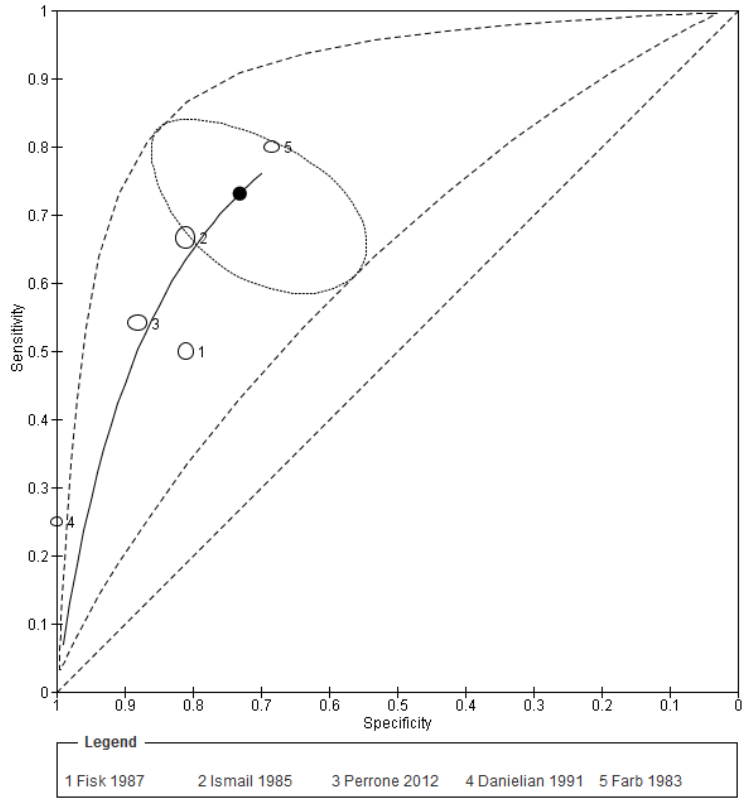
estimates for these studies are: sensitivity 59% (95%CI 48-69%) and specificity 83% (95% CI 74-89%), Likelihood Ratio positive, LR+ 3.45 (95%CI 2.24-5.30) and Likelihood Ratio negative LR- 0.50 (95%CI 0.38-0.64). The SROC plot for these studies is shown in Figure 7b.

Figure 7a. Sensitivity and Specificity for studies evaluating C-Reactive Protein in the Diagnosis of and/or Funisitis at a cut-off of 20mg/L



TP- True Positive, FP- False Positive, FN- False Negative, TN- True Negative, CI- Confidence Interval.

Figure 7b. Summary ROC curve for studies evaluating C-Reactive Protein for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis at a cut-off of 20mg/L.

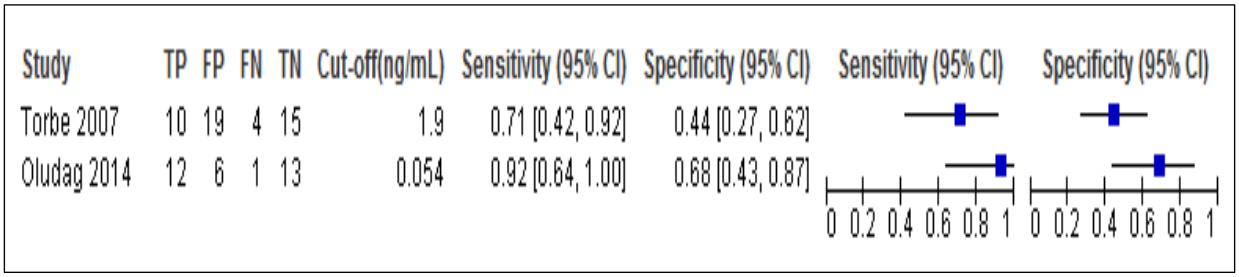


• Summary Point; ⬭ 95% confidence region; ⬭ - 95% prediction region.

Studies Evaluating Procalcitonin in the Diagnosis of Histologic Chorioamnionitis and/or Funisitis

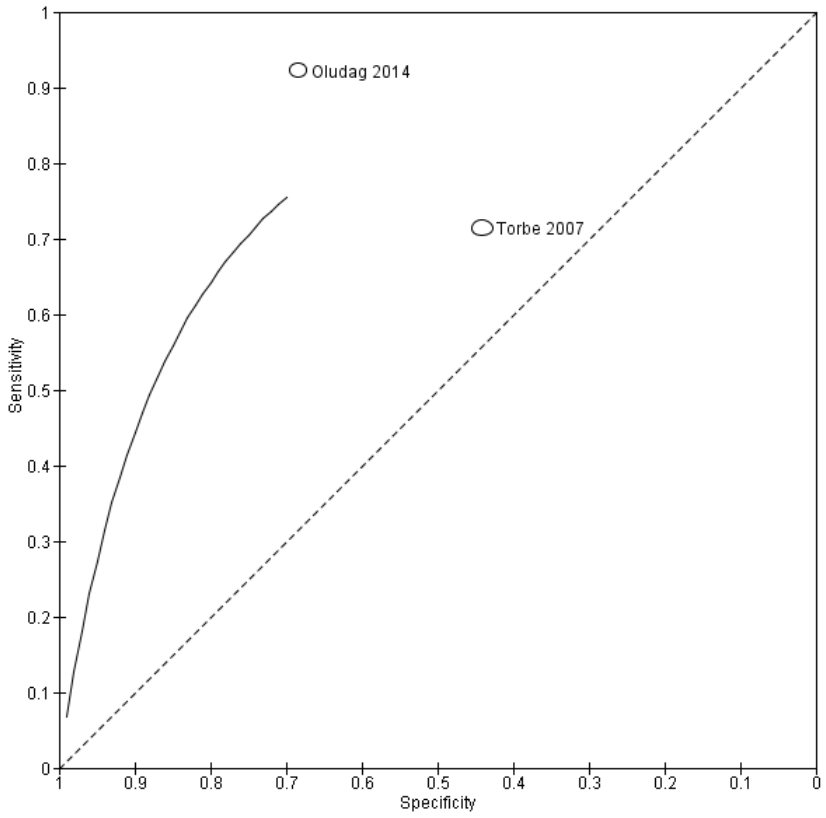
We included 2 studies in this category: Oludag *et al*[115] who used a cut-off of 1.9ng/ml and Torbe *et al*[56] who used a cut-off of 0.054ng/ml. Both studies assessed HCA as the reference standard. The sensitivity and specificity of these studies and their 95% CI are shown in Figure 8a and are plotted in ROC space in Figure 8b. No summary estimate is shown as these studies used different cut-offs.

Figure 8a. Forest Plot Showing Sensitivity and Specificity for Studies Evaluating Procalcitonin in the Diagnosis of Histologic Chorioamnionitis and/or Funisitis



TP- True Positive, FP- False Positive, FN- False Negative, TN- True Negative, CI- Confidence Interval.

Figure 8b. Sensitivity and Specificity for Studies Evaluating Procalcitonin for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis plotted in ROC space.

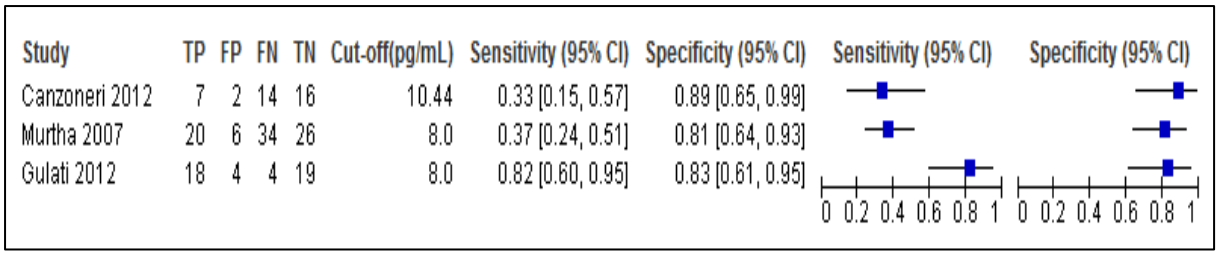


Studies Evaluating Interleukin 6 in the Diagnosis of Histologic Chorioamnionitis and/or Funisitis

We included 3 studies assessing the marker IL6. Murtha *et al*[120] and Canzoneri *et al*[123] assessed Funisitis and Gulati *et al*[114] assessed HCA as the reference standard. Canzoneri *et al*[123] used the cut-offs of 1.98, 5.12 and 10.44 pg/mL. Murtha *et al*[120] used cut-offs of 1.8pg/mL and 8pg/mL. We selected the commonly used cut-off of 8pg/mL. For the Canzoneri *et al*[123] study, we used the cut-off closest to 8pg/mL, that is 10.44pg/mL.

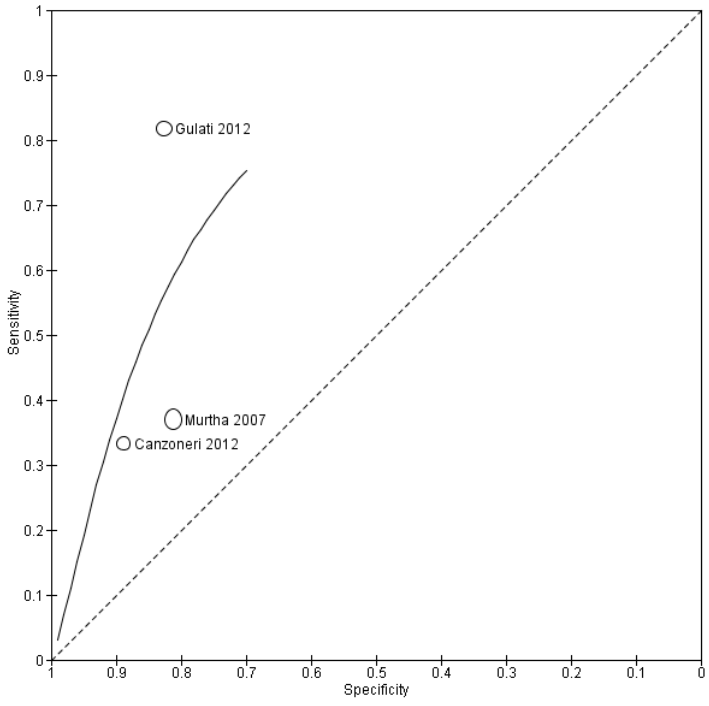
The sensitivity and specificity of these studies and their 95% CIs are shown in Figure 9a and are plotted in ROC space in Figure 9b. No summary estimate is shown as these studies used different cut-offs.

Figure 9a. Forest Plot Showing Sensitivity and Specificity for Studies Evaluating IL6 in the Diagnosis of Histologic Chorioamnionitis and/or Funisitis



TP- True Positive, FP- False Positive, FN- False Negative, TN- True Negative, CI- Confidence Interval.

Figure 9b. Sensitivity and Specificity for Studies Evaluating Interleukin 6 for the Diagnosis of Histologic Chorioamnionitis and/or Funisitis plotted in ROC space.



Investigations for Heterogeneity for the Diagnostic Review

Investigations for heterogeneity were conducted only for CRP studies as the other 2 index tests had insufficient number of studies to perform objective investigations for heterogeneity.[105,106]

Visual inspection of forest plots (Figure 6a) and ROC plots (Figure 6b) revealed marked variability in the estimates of sensitivity and specificity from the various studies. The 95% prediction region of the SROC curve was very large indicating high heterogeneity (see Figure 6b). The prediction region was smaller when the studies were limited to those using the same cut-off (20mg/L) (Figure 7b) indicating that some of the heterogeneity was due to the differences in cut-offs. However, the 95% prediction region of this curve (Figure 7b) was still large indicating heterogeneity remained after accounting for effects of the different thresholds.

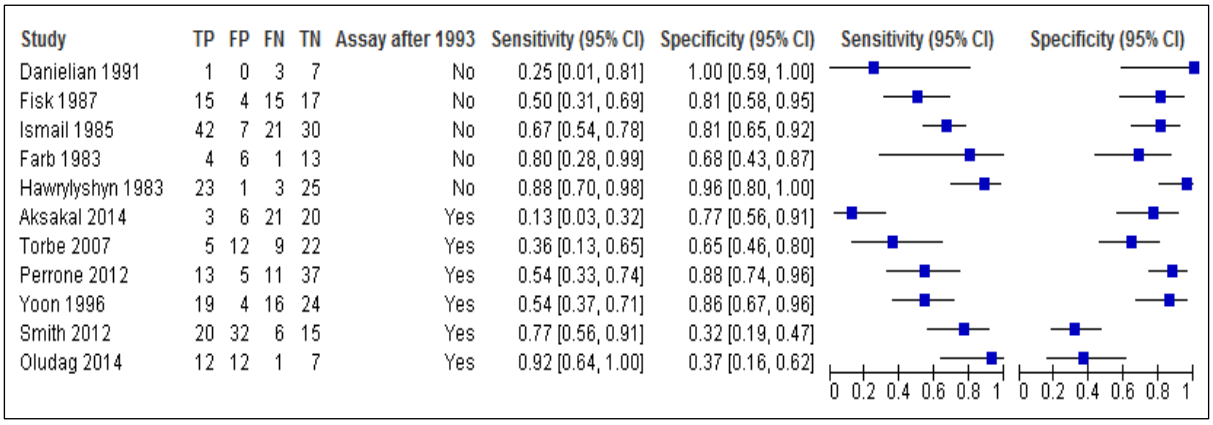
Planned investigation for heterogeneity due to differences in antibiotic use was not conducted due to poor reporting of antibiotic use. Where antibiotics were used selectively, the proportion of patients who received it was not reported. Investigations for heterogeneity were therefore conducted only on the following characteristics

(i) Assay type
(ii) Pre-specified threshold
(iii) Interval between sampling time and delivery
(iv) Risk of Bias in the patient selection domain

Assay type

The assay type (see Table 5) for the CRP assays was investigated as a possible source of heterogeneity. Standardisation for CRP assays was first performed in 1993[132]. We grouped the studies into 2 according to the year the study was conducted as a proxy for CRP before standardisation and after standardisation with 1993 as the cut-off. Five studies[118,125–128] were conducted before 1993 and 6 studies[56,115,117,119,121,124] at or after 1993. Figure 10a shows the corresponding sensitivities and specificities of the studies in the subgroups. No pooled estimates were obtained for the 2 subgroups due to differences in cut-offs.

Figure 10a. Sensitivity and Specificity for studies evaluating C-Reactive Protein in the Diagnosis of and/or Funisitis, Subgroups: Assays Performed Before and After 1993.

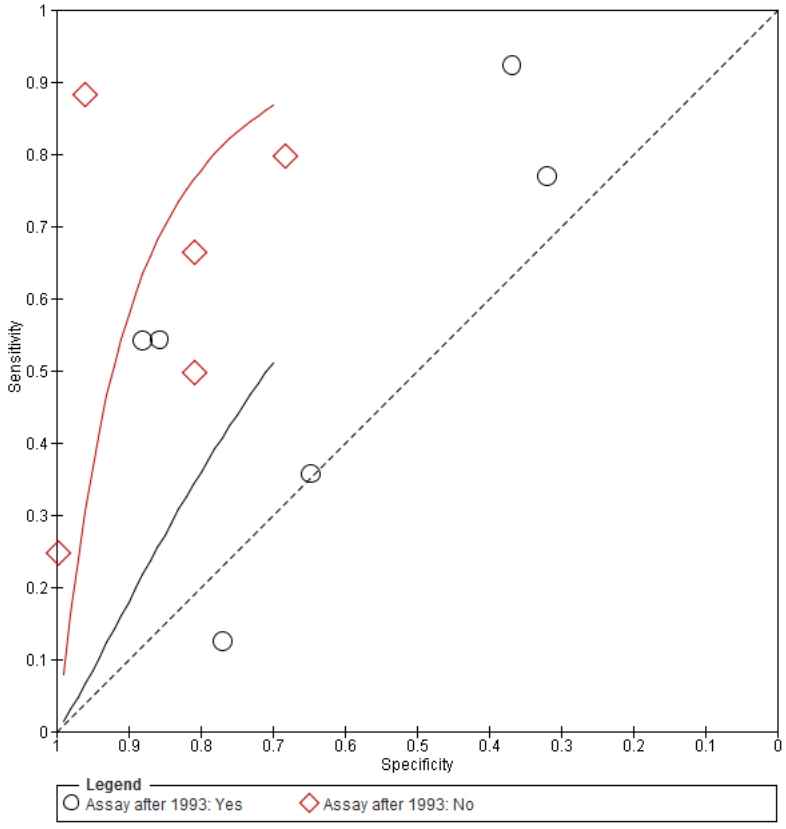


Study	TP	FP	FN	TN	Assay after 1993	Sensitivity (95% CI)	Specificity (95% CI)
Danielian 1991	1	0	3	7	No	0.25 [0.01, 0.81]	1.00 [0.59, 1.00]
Fisk 1987	15	4	15	17	No	0.50 [0.31, 0.69]	0.81 [0.58, 0.95]
Ismail 1985	42	7	21	30	No	0.67 [0.54, 0.78]	0.81 [0.65, 0.92]
Farb 1983	4	6	1	13	No	0.80 [0.28, 0.99]	0.68 [0.43, 0.87]
Hawrylyshyn 1983	23	1	3	25	No	0.88 [0.70, 0.98]	0.96 [0.80, 1.00]
Aksakal 2014	3	6	21	20	Yes	0.13 [0.03, 0.32]	0.77 [0.56, 0.91]
Torbe 2007	5	12	9	22	Yes	0.36 [0.13, 0.65]	0.65 [0.46, 0.80]
Perrone 2012	13	5	11	37	Yes	0.54 [0.33, 0.74]	0.88 [0.74, 0.96]
Yoon 1996	19	4	16	24	Yes	0.54 [0.37, 0.71]	0.86 [0.67, 0.96]
Smith 2012	20	32	6	15	Yes	0.77 [0.56, 0.91]	0.32 [0.19, 0.47]
Oludag 2014	12	12	1	7	Yes	0.92 [0.64, 1.00]	0.37 [0.16, 0.62]

TP- True Positive, FP- False Positive, FN- False Negative, TN- True Negative, CI- Confidence Interval.

The SROC plots for the two subgroups are shown in Figure 10b and reflect differences in diagnostic accuracy. The -2 log likelihoods of the 2 plots were compared with the χ^2 test yielding a p value of 0.086.

Figure 10b. SROC Plots Comparing Studies Evaluating CRP in the Diagnosis of HCA and/or Funisitis, Subgroups Assays Performed Before and After 1993.

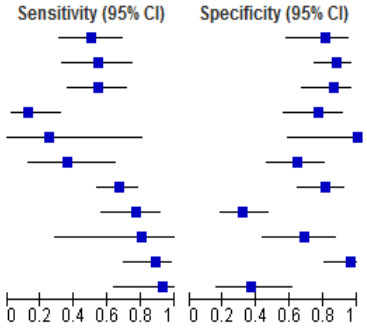


Pre-specified cut-off

Studies were grouped according to whether the cut-off used was pre-specified or whether it was determined from the study data. This was an aspect of quality assessment that was judged in the 'Index Test' domain of the QUADAS-2[102] tool. Figure 11a shows Sensitivity and Specificity in the 2 subgroups. No pooled estimates were obtained for the 2 subgroups due to differences in cut-offs.

Figure11a. Sensitivity and Specificity for studies evaluating C-Reactive Protein in the Diagnosis of and/or Funisitis, Subgroups: Pre-specified Cut-off or Not

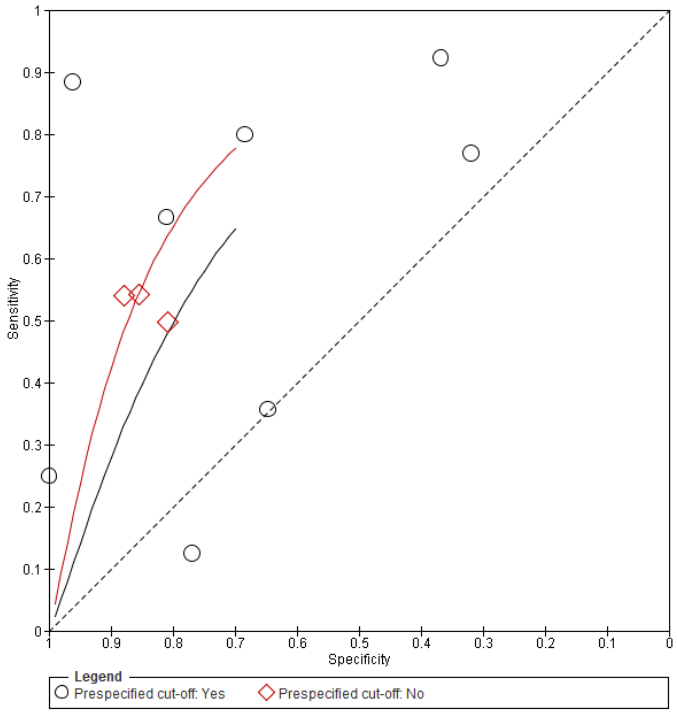
Study	TP	FP	FN	TN	Prespecified cut-off	Sensitivity (95% CI)	Specificity (95% CI)
Fisk 1987	15	4	15	17	No	0.50 [0.31, 0.69]	0.81 [0.58, 0.95]
Perrone 2012	13	5	11	37	No	0.54 [0.33, 0.74]	0.88 [0.74, 0.96]
Yoon 1996	19	4	16	24	No	0.54 [0.37, 0.71]	0.86 [0.67, 0.96]
Aksakal 2014	3	6	21	20	Yes	0.13 [0.03, 0.32]	0.77 [0.56, 0.91]
Danielian 1991	1	0	3	7	Yes	0.25 [0.01, 0.81]	1.00 [0.59, 1.00]
Torbe 2007	5	12	9	22	Yes	0.36 [0.13, 0.65]	0.65 [0.46, 0.80]
Ismail 1985	42	7	21	30	Yes	0.67 [0.54, 0.78]	0.81 [0.65, 0.92]
Smith 2012	20	32	6	15	Yes	0.77 [0.56, 0.91]	0.32 [0.19, 0.47]
Farb 1983	4	6	1	13	Yes	0.80 [0.28, 0.99]	0.68 [0.43, 0.87]
Hawrylyshyn 1983	23	1	3	25	Yes	0.88 [0.70, 0.98]	0.96 [0.80, 1.00]
Oludag 2014	12	12	1	7	Yes	0.92 [0.64, 1.00]	0.37 [0.16, 0.62]



TP- True Positive, FP- False Positive, FN- False Negative, TN- True Negative, CI- Confidence Interval.

The SROC plots for the two subgroups are shown in Figure 11b. The -2 log likelihoods of the 2 plots were compared with the χ^2 test, p=0.472, indicating no evidence for a difference in the two plots. The 3 studies that did not use pre-specified cut-offs had less variable sensitivity and specificity compared to the other studies.

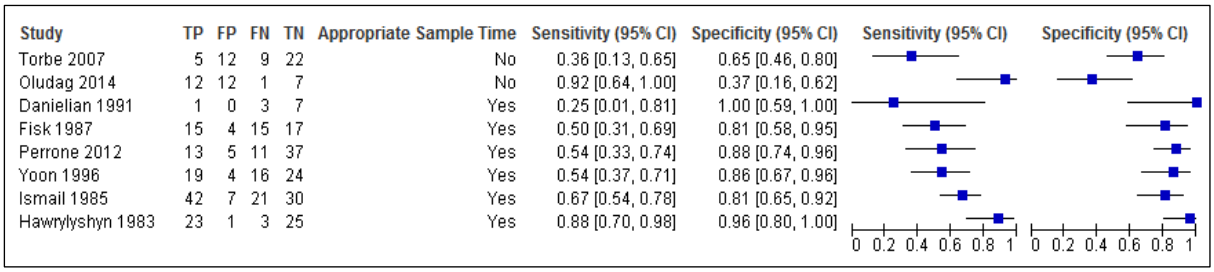
Figure 11b. SROC Plots Comparing Studies Evaluating CRP in the Diagnosis of HCA and/or Funisitis, Subgroups: Pre-specified cut-off or not.



Interval from sampling to delivery

Studies were grouped according to sampling time with an interval of 72hours between sampling and delivery as the cut-off. Four studies[114,117,124,125] were excluded from this analysis due to unclear interval. This was an aspect of quality assessment that was judged in the 'Flow and timing' domain of the QUADAS-2[102] tool. Figure 12a shows the corresponding sensitivities and specificities in the 2 subgroups. No pooled estimates were obtained for the subgroups due to differences in cut-offs.

Figure 12a. Sensitivity and Specificity for studies evaluating C-Reactive Protein in the Diagnosis of and/or Funisitis, Subgroups: Appropriate Sample Interval or Not

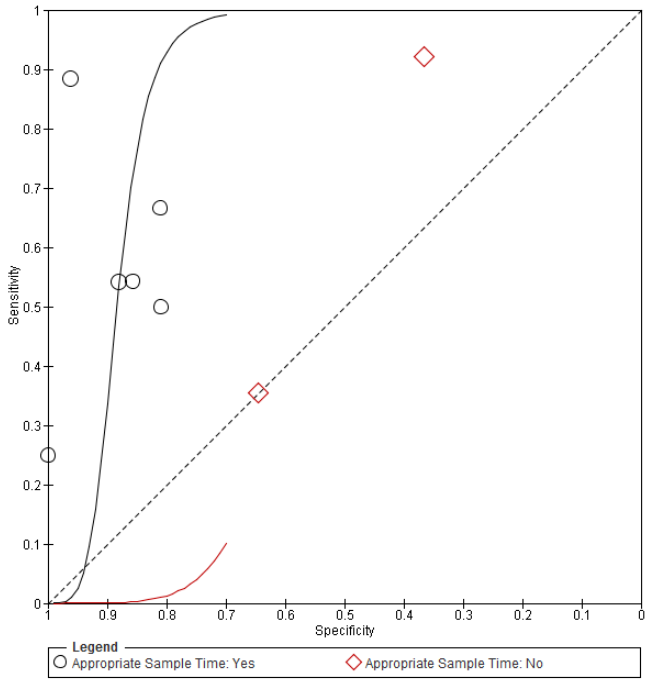


TP- True Positive, FP- False Positive, FN- False Negative, TN- True Negative, CI- Confidence Interval.

Appropriate sample time - ≤72hours

The SROC plots for the two subgroups are shown in Figure 12b. The -2 log likelihoods of the 2 plots were compared with the χ^2 test. p=0.005 indicating that the 2 plots are different.

Figure 12b. SROC Plots comparing Studies Evaluating CRP in the Diagnosis of HCA and/or Funisitis, Subgroups: Appropriate Sample Interval* or Not



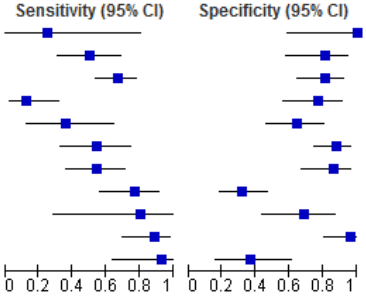
*Appropriate sample time - ≤72hours

Risk of Bias in Patient Selection

The judgements on risk of bias in the patient selection domain of the QUADAS-2[102] tool were used to classify studies into 2 subgroups: high risk and low risk. Figure 13a shows the corresponding sensitivities and specificities in the 2 subgroups. No pooled estimates were obtained for the subgroups due to differences in cut-offs.

Figure 13a. Sensitivity and Specificity for studies evaluating C-Reactive Protein in the Diagnosis of and/or Funisitis, Subgroups: Low Risk and High Risk of Bias in the Patient Selection Domain

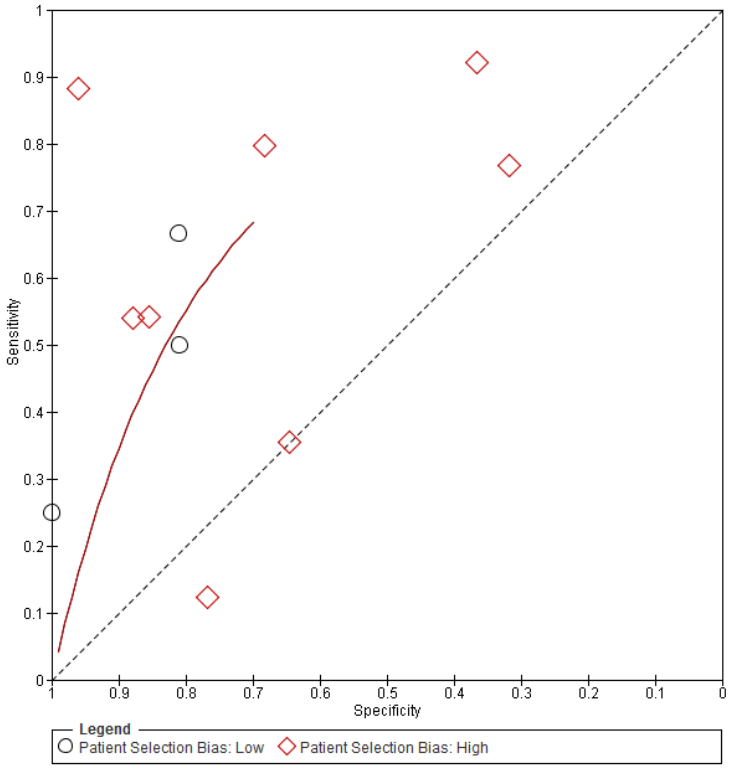
Study	TP	FP	FN	TN	Patient Selection Bias	Sensitivity (95% CI)	Specificity (95% CI)
Danielian 1991	1	0	3	7	Low	0.25 [0.01, 0.81]	1.00 [0.59, 1.00]
Fisk 1987	15	4	15	17	Low	0.50 [0.31, 0.69]	0.81 [0.58, 0.95]
Ismail 1985	42	7	21	30	Low	0.67 [0.54, 0.78]	0.81 [0.65, 0.92]
Aksakal 2014	3	6	21	20	High	0.13 [0.03, 0.32]	0.77 [0.56, 0.91]
Torbe 2007	5	12	9	22	High	0.36 [0.13, 0.65]	0.65 [0.46, 0.80]
Perrone 2012	13	5	11	37	High	0.54 [0.33, 0.74]	0.88 [0.74, 0.96]
Yoon 1996	19	4	16	24	High	0.54 [0.37, 0.71]	0.86 [0.67, 0.96]
Smith 2012	20	32	6	15	High	0.77 [0.56, 0.91]	0.32 [0.19, 0.47]
Farb 1983	4	6	1	13	High	0.80 [0.28, 0.99]	0.68 [0.43, 0.87]
Hawrylyshyn 1983	23	1	3	25	High	0.88 [0.70, 0.98]	0.96 [0.80, 1.00]
Oludag 2014	12	12	1	7	High	0.92 [0.64, 1.00]	0.37 [0.16, 0.62]



TP- True Positive, FP- False Positive, FN- False Negative, TN- True Negative, CI- Confidence Interval.

The SROC plots for the two subgroups are shown in Figure 13b. Visually, the 2 plots overlapped. The -2 log likelihoods of the 2 plots were compared with the $_{X}^{2}$ test. p=0.951 indicating no evidence for a difference in the 2 plots.

Figure 13b. SROC Plots comparing Studies Evaluating CRP in the Diagnosis of HCA and/or Funisitis, Subgroups: Low Risk and High Risk of Bias in the Patient Selection Domain



Sensitivity Analysis

Gestational Age

We investigated whether using a narrower gestational age range for inclusion to the review would alter the findings of the review. We constructed an SROC plot including all studies regardless of gestational age range. We then constructed a second SROC plot excluding studies that had gestational age whose lower limit included gestations less than 24 weeks (4 studies)[117,119,125,128] The cut-off of 24 weeks was chosen as this is the gestation at which the foetus is considered to be viable.[133] The two SROC plots are shown in Figure 14 a and b. There was little difference in the shape and accuracy of the two plots.

Figure 14. Sensitivity Analysis for Gestational Age in Studies Evaluating CRP in the Diagnosis of HCA/Funisitis.

Figure 14a. All included studies

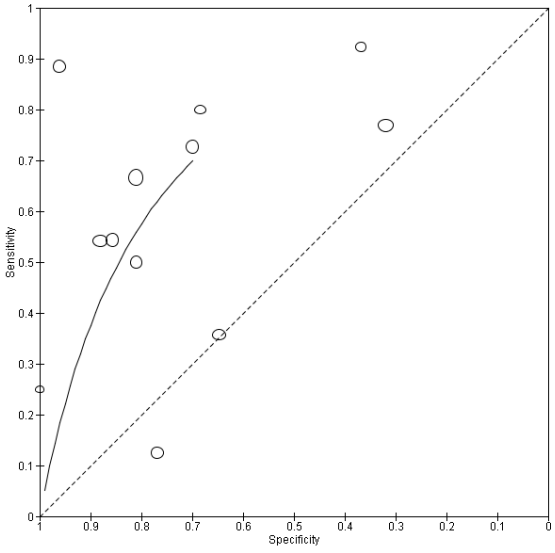
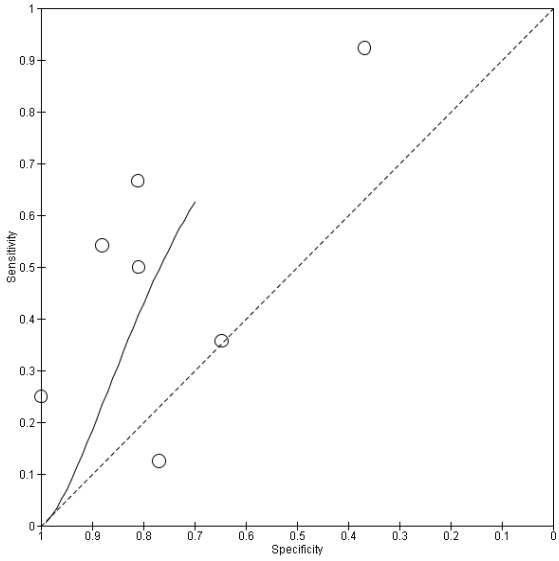


Figure 14b. Studies without early (<24 weeks) gestations.



Applicability Concerns in Patient Selection

We investigated whether excluding studies that had high concerns for applicability to the review in the patient selection domain of QUADAS-2[102] would change the findings of the review. We constructed an SROC plot that included all studies. We then constructed a second SROC plot excluding studies that had high concerns for applicability.[117,119,121,125–128] The two SROC plots are shown in figure 15a and b. The 2 plots differed in both shape and accuracy with a reduction in accuracy after exclusion of studies with high concern for applicability.

Figure 15. Sensitivity Analysis for Applicability Concerns in Patient Selection in Studies Evaluating CRP in the Diagnosis of HCA/Funisitis.

Figure 15a. All included studies

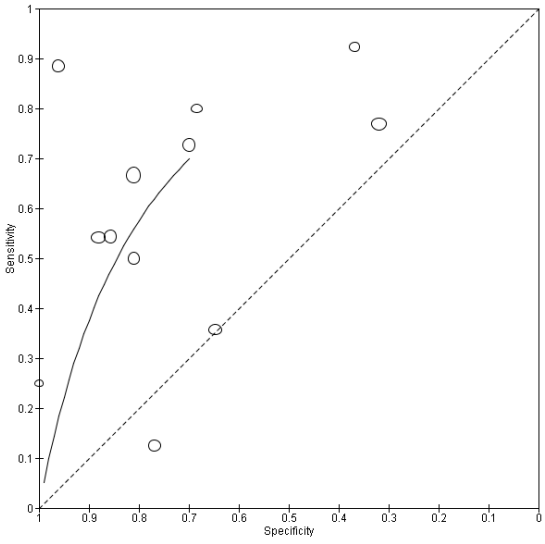
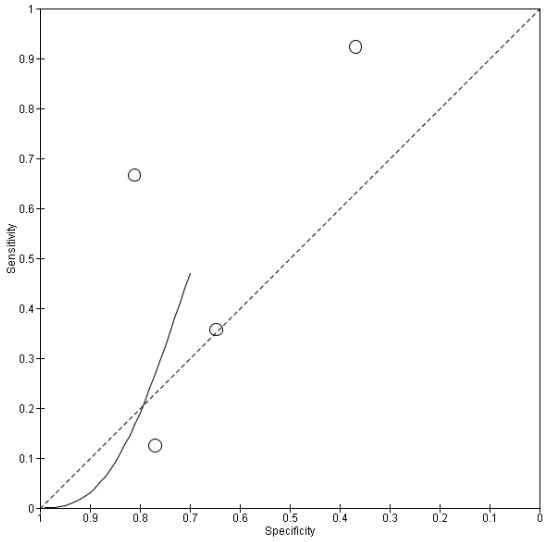


Figure 15b. Studies with low applicability concerns in patient selection.



Year of Publication

We investigated whether excluding studies that were published more than 15 years prior to the review search date (2015) would alter the findings of the review. We constructed an SROC plot that included all studies. We then constructed a second SROC plot excluding studies published before 2000.[118,119,125–128] The two SROC plots are shown in figure 16a and b. They show that limiting the review to studies published in the preceding 15 years would likely result in a lower accuracy.

Figure 16. Sensitivity Analysis for Year of Publication in Studies Evaluating CRP in the Diagnosis of HCA/Funisitis.

Figure 16a. All included studies

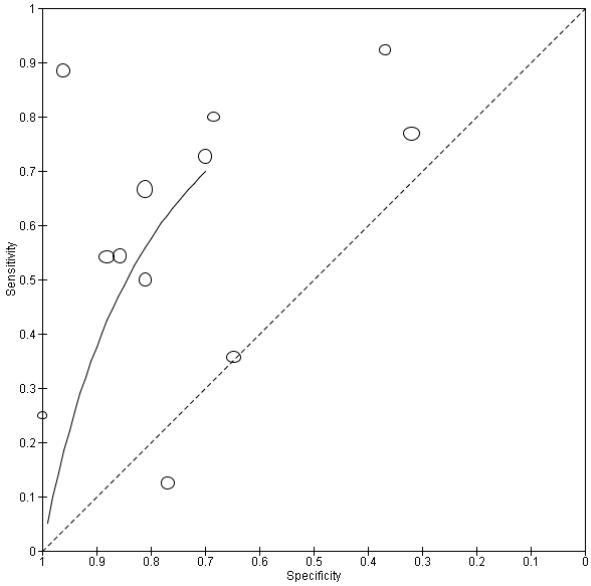
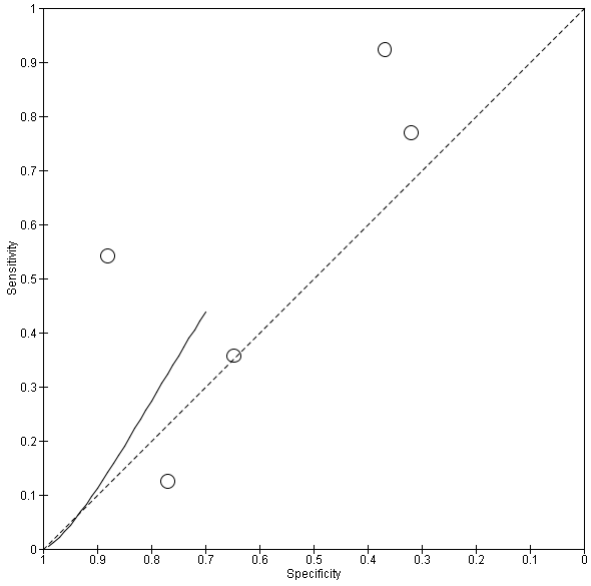


Figure 16b. Studies published within 15 years of the review search date.



Inflammatory markers for prediction of Early Onset Neonatal Sepsis

Characteristics of Included Studies

In this section of the review, we included 7 studies conducted across 5 countries. These studies were completed between 2001 and 2009 and published between 2005 and 2014. All studies were in inpatient settings. In total, 356 pregnancies with PPROM were included with data reported for 332 pregnancies and 97 episodes of EONS, Median prevalence 26% (Range 19-44%, IQR 26-34%). Majority of the studies (6/7) were prospective cohort designs with 1 study[61] of retrospective cohort design. Characteristics of the included studies for this section of the review are given in Table 6.

Characteristics of included patients

All studies had no restrictions on maternal age or parity. The lower limit for gestational age for inclusion to the studies was 24weeks in all but 1 study. One study[61] did not explicitly report the gestational age range but only specified 'preterm' gestational age. The method of gestational age assessment was not reported in 3 studies.[61,114,115] Last menstrual period and confirmation by ultrasound was used in the remaining 4 studies.

Management of PPROM in mothers

All except one study[129] reported use of antenatal (maternal) antibiotics and steroids in all or most of the included patients. Use of tocolytics was reported in only 1 study[61] where tocolytics were administered in 78% of included patients. Reasons for delivery were reported in only 1 study[114] and these included: gestation greater than 34 weeks, signs and symptoms of clinical chorioamnionitis, non-reassuring foetal heart rate pattern and other obstetric complications.

Management of the neonates

Only 2 studies reported management of the neonates born to mothers with PPROM. Torbe *et al*[40] stated that 'all infected new-borns received antibiotics after delivery'. Hatzidaki *et al*[61] outlined the management of neonates: 'all neonates were admitted, underwent clinical and laboratory evaluation for sepsis and were consequently administered empiric antibiotics.'

Reference Standard

Studies used various definitions and timelines for the outcome (see table 4). Three studies used a timeline of 48 hrs after birth[40,56,130] and 1 study used a timeline of 72 hours.[115] Two studies[61,114] used the word 'early' to define the timeline with one of them[61] specifying a duration of positive blood culture of 4 days from birth. Kayem *et al*[129] did not specify a

timeline but indicated that new-borns were evaluated with results of tests being available within 1 hour. There were differences in the methods of ascertainment of the outcome. Five studies used a combination of clinical and laboratory features.[40,56,61,129,130] Oludag *et al* [115] used only laboratory criteria to ascertain the outcome. Gulati *et al*[114] did not give details of the methods but simply specified 'early neonatal sepsis'. The reference standard from all included studies shall thence be referred to as Early Onset Neonatal Sepsis (EONS).

Index tests

Of the 7 included studies, 5 assessed CRP as the index test, 2 assessed PCT and 2 assessed IL6. Details of the test assays are provided in Table 5.

Table 6. Characteristics of included studies, Index Test for Early Onset Neonatal Sepsis

Study	Country	Study Design	No of Participants(excluded*)	GA Range Criteria (weeks)	Actual GA at admission or at ROM (weeks)	GA at delivery (weeks)	Time from ROM to delivery	Antibiotics	Steroids	Tocolytics	Outcome	Prevalence of outcome (%)
Kayem 2005[129]	France	Prospective cohort	75(2 neonatal deaths)	24-34	Mean ±SD 28.4±3.2	Mean ±SD 31.2±3.2	NR	NR	NR	NR	Neonatal Infection Probable or proven Both clinical and lab evaluation Assessment at birth	14/73(19%)
Hatzidaki 2005[61]	Greece	Retrospective cohort	58(0)	'Preterm'	NR	Mean ±SD, 32.6 ±2.9	Mean ±SD, With sepsis 293.3±90.4, Without sepsis 154.2±48.3 hours	Selective (81%)	Selective (81%)	Selective (77.6%)	Early sepsis Positive blood culture within the first 4 days of life Both suspected and confirmed sepsis Clinical and lab evaluation	20/58 (34%)
Torbe 2007[56]	Poland	Prospective Cohort	48(0)	24 to 34	Mean ±SD, 30.8 ±3.3 (at ROM)	Mean ±SD, 31.4±3.0	Mean ±SD, 5.5± 8.1 days	Yes	Yes	None	Perinatally acquired neonatal infection Within 48hours of delivery Clinical signs and laboratory features	17/48 (35%)
Torbe 2010[40]	Poland	Prospective cohort	50(0)	24 to 36	Mean ±SD, Neonates with infection 30.9±3.6, No infection 32.5±3.5	NR	Mean ±SD, Neonates with infection 4.4 ±6.6, Without infection 3.0 ±4.8 days	Yes (all)	Selective, < 34 weeks	NR	Early onset neonatal infection <48 hours after delivery Both proven and suspected Clinical signs and microbial status	14/50 (28%)
Torbe 2011[130]	Poland	Prospective cohort	48(17, no data available	28-35	Mean ± SD, 32.3±2.63	Mean ±SD, 32.4±2.57	Mean ±SD, 4.06±4.62 days	yes	yes	NR	Perinatally acquired neonatal infection Within 48hours of delivery Clinical signs and laboratory features	8/31 (26%)
Gulati 2012[114]	India	Prospective Cohort	45(5 stillbirths	24 to 34	Mean ±SD, 30.5±2.13	NR	NR	Yes	Yes	NR	Early neonatal sepsis	10/40 (25%)
Oludag 2014[115]	Turkey	Prospective Cohort	32(0)	24 to 34	Mean ±SD, 28.1±3.3	NR	NR	Yes	Yes	NR	Neonatal infection Positive blood culture after 72 hours and CRP levels	14/32 (44%)
Totals			356(24)									97/332 (29%)

*Number excluded from analysis, with reasons; SD, Standard Deviation; NR, Not Reported;

Methodological Quality of Included Studies

Risk of Bias in Included Studies

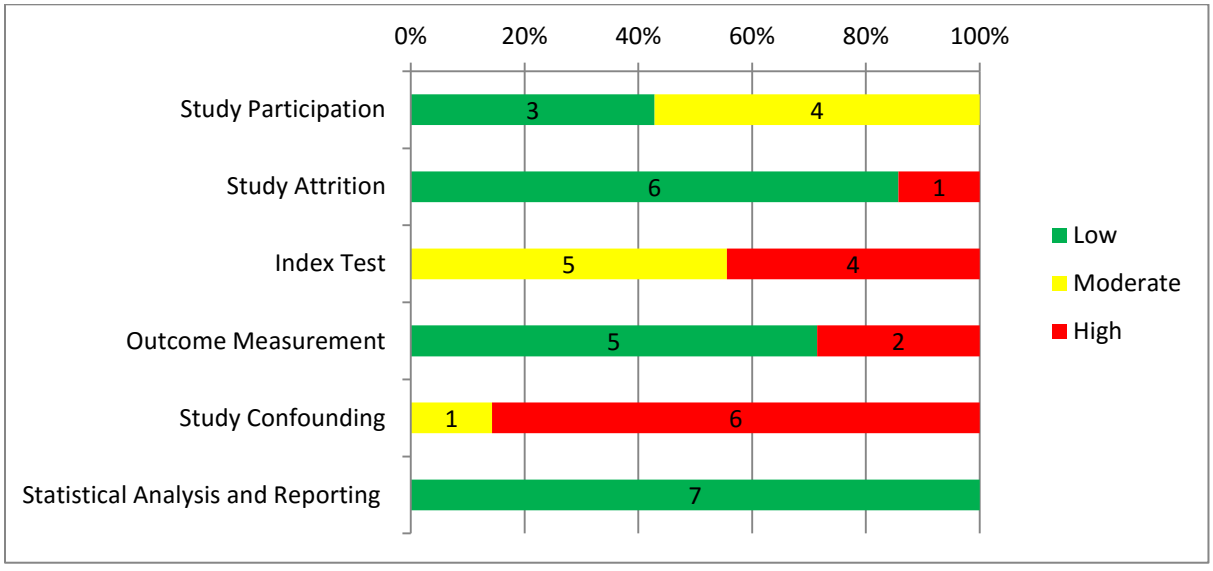
We used the QUIPS[103] tool to assess the risk of bias in the 7 included studies. Individual study judgements for risk of bias are given in Table 7. A summary of the risk of bias judgements for the studies is shown in Figure 17.

Table 7. Individual Study Judgements for Risk of Bias for Studies Evaluating Inflammatory Markers in the Prediction of EONS.

Study	Study Participation	Study Attrition	Index Test: CRP	Index Test: PCT	Index Test: IL6	Outcome Measurement	Study Confounding	Statistical Analysis and Reporting
Kayem 2005[129]	Low	Low	High			Low	Moderate	Low
Hatzidaki 2005[61]	Low	Low			High	Low	High	Low
Torbe 2007[56]	Low	Low	Moderate	High		Low	High	Low
Torbe 2010[40]	Moderate	Low	Moderate			Low	High	Low
Torbe 2011[130]	Moderate	High	Moderate			Low	High	Low
Gulati 2012[114]	Moderate	Low			Moderate	High	High	Low
Oludag 2014[115]	Moderate	Low	Moderate	High		High	High	Low

CRP, C reactive protein; PCT, Procalcitonin; IL6, Interleukin 6.

Figure 17. Summary of the Risk of Bias for Studies Evaluating Inflammatory Markers in the Prediction of EONS.



All studies were judged to be at high risk of bias in at least 1 of the 6 domains. The domain with the poorest assessment was that of 'Study Confounding'. In this domain, only 1 study[129] was judged to be at moderate risk of bias with all the others judged to be at high risk of bias. Two studies [61,129] reported measurement of potential confounders. Hatzidaki *et al*[61] measured possible confounders and performed logistic regression for the index test and other factors in predicting early sepsis. However, the results of the logistic regression were not completely reported and it was not possible to extract measures of association/effect adjusted for confounders. This study was therefore also judged to be at high risk of bias in this domain. Kayem *et al*[129] also measured some potential confounders, and performed logistic regression with variables such as gestation, white blood cell count and vaginal fluid IL6 positivity. They reported crude ORs for the association and adjusted OR adjusted for the other significant variable – vaginal IL6 levels. This study was judged to be at moderate risk of bias due to partial accounting for confounders in the analysis.

The 'Index Test' domain also performed poorly. Some studies used cut-offs obtained from study data rather than predetermined cut-offs (see Table 5) and were therefore deemed to be at high risk of bias in this domain. While several studies[40,56,115,130] reported a constant sampling time relative to the time of ROM or to the time of admission, the interval relative to the time of delivery varied due to the different durations of time from ROM to delivery (latency) among study participants. A constant time interval relative to the time of delivery would be preferred to enable a consistent relationship between maternal blood sampling and delivery and by extension development of neonatal sepsis. In some studies[40,122] samples were obtained at several points during latency but it was not clear which sample was used for the analysis. Studies with unclear or inconsistent sampling time relative to delivery were judged to be at moderate risk of bias. The laboratory methods for the index tests were well reported and deemed reliable in all but 1 study.[129] This study provided no details of the assays and procedures for the test and was therefore judged to be at high risk of bias. All studies carried out the laboratory analysis for all study participants in the same way.

In the patient selection domain, some studies had exclusions which were deemed inappropriate.[40,114,115,130] These studies excluded patients with what was reported as 'any maternal or foetal complications', a feature which would result in a study population with different characteristics from the usual patient population with PPROM. These studies were judged to be at moderate risk of bias.

Only 1 study[130] was deemed to be at high risk of bias in the study attrition domain. In this study, data were reported for only 65% of patients with no elaboration on reasons for the missing data.

Two studies[114,115] were judged to be at high risk of bias in the study outcome domain. This was due to insufficient definitions of the outcome of interest. Gulati *et al*[114] simply stated the outcome as 'early neonatal sepsis'. Oludag *et al*[115] used a criteria that relied only on laboratory features for outcome ascertainment with no incorporation of clinical features.

In all studies, it was possible to extract or calculate 2x2 tables for the index test and the outcome and all studies were therefore judged to be at low risk of bias in this domain. Most studies calculated sensitivity and specificity for the index test as a diagnostic/ predictive factor.

Applicability concerns

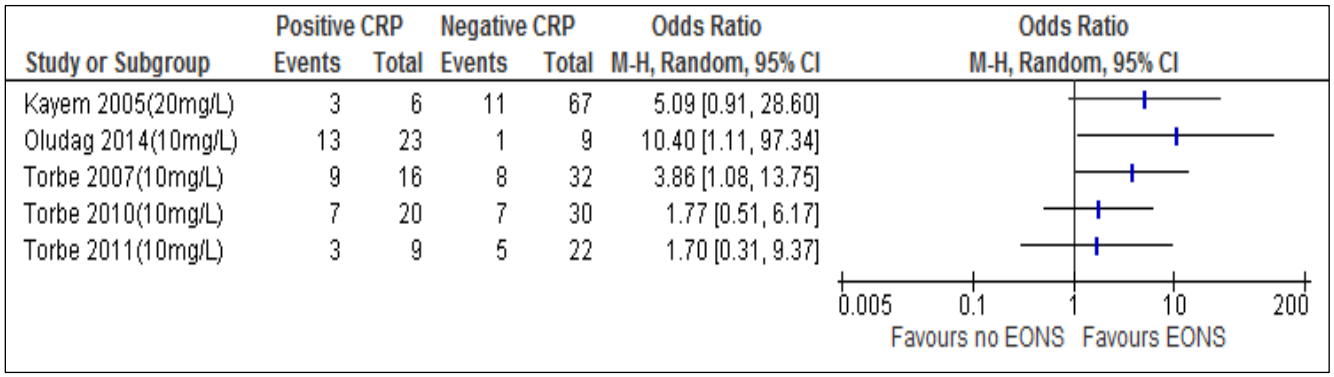
No standardised assessment of applicability of the included studies to the review was carried out. However, we noted significant concerns in the methods of ascertainment of the outcome. The included studies had different definitions of infection in the early neonatal period some of which may not be a reliable match for the outcome of interest of the review. Studies where the definition of infection relied only on laboratory features[115] and studies where the duration post-delivery was not clearly indicated[114] were considered to have high concerns for applicability. In addition, clinical and laboratory protocols for how and when neonatal assessments and investigations are carried out were poorly reported and may have differed in the included studies.

Findings

Studies Evaluating the Role of C-Reactive Protein in Prediction of EONS

There were 5 included studies assessing the role of CRP. Four studies used a cut-off of 10mg/L. One study[40] also assessed the outcomes against a cut off of 15mg/L. Another study[129] assessed the outcome against two cut-offs: 5mg/L and 20mg/L but 2x2 data was available for the 20mg/L cut-off only. Individual study ORs (unadjusted) are provided in Figure 18. We did not pool ORs from the 5 studies due to use of a different cut-off in one study.[129]

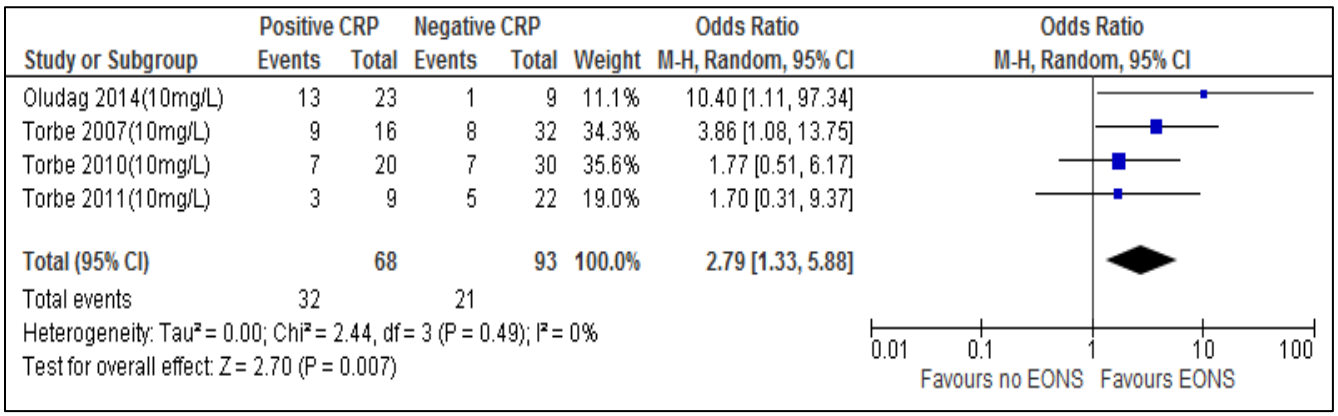
Figure 18. Forest Plot Showing Individual Study Odds Ratios (Unadjusted) for Studies Evaluating CRP at all cut-offs in prediction of EONS.



CRP, C reactive protein; MH, Mantel-Haenszel; EONS, Early Onset Neonatal Sepsis; CI, Confidence Interval.

We limited further analysis to the 4 studies that reported data at a cut-off of 10mg/L. Individual and pooled unadjusted ORs for these 4 studies are shown in Figure 19. The pooled unadjusted OR for the 4 studies was 2.79 (95% CI 1.33 – 5.88). Chi squared test was used to test for statistical significance obtaining a p of 0.007 for the overall effect. Statistical heterogeneity was low, I^2 = 0%, p 0.490.

Figure 19. Forest Plot Showing Individual Study and Pooled Odds Ratios (Unadjusted) for Studies Evaluating CRP at 10mg/L in prediction of EONS.

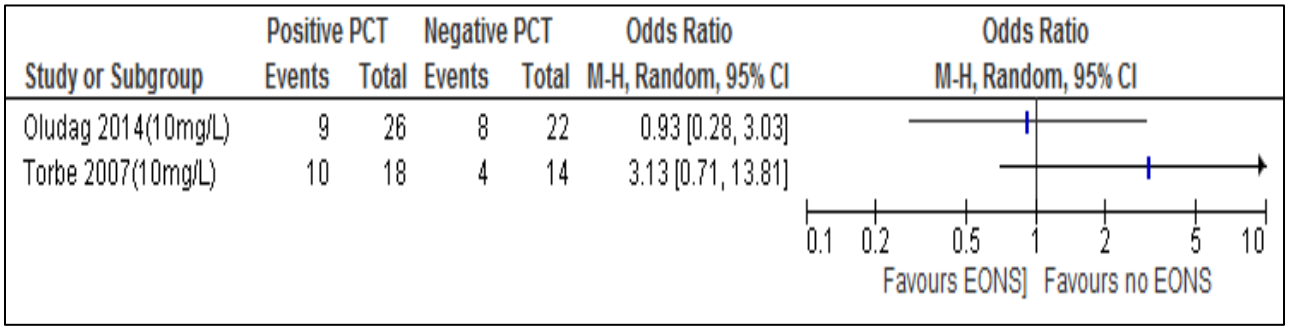


CRP, C reactive protein; MH, Mantel-Haenszel; EONS, Early Onset Neonatal Sepsis; CI, Confidence Interval.

Studies Evaluating the Role of Procalcitonin in Prediction of EONS

We included 2 studies assessing PCT. Two cut-offs were used: 1.9ng/mL[115] and 0.054ng/mL.[56] The individual unadjusted ORs of the 2 studies are shown in Figure 20. We did not pool ORs for these studies as they used different cut-offs.

Figure 20. Forest Plot Showing Individual Study Odds Ratios (Unadjusted) for Studies Evaluating Procalcitonin at all cut-offs in prediction of EONS.

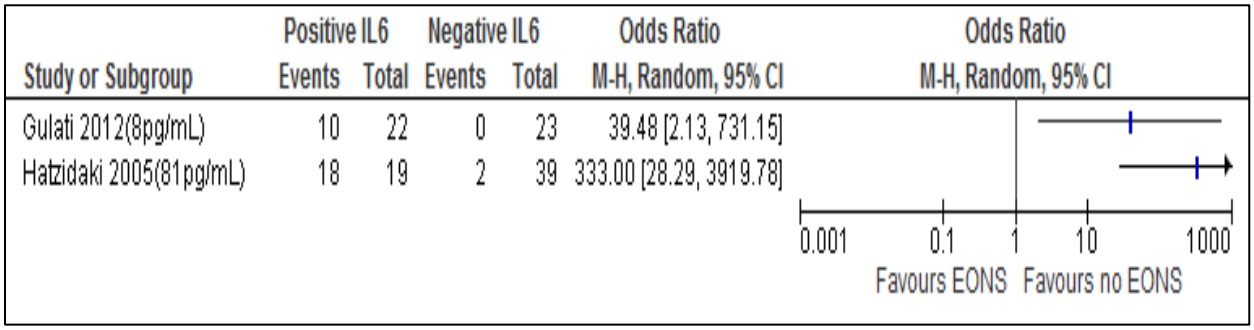


PCT, Procalcitonin; MH, Mantel-Haenszel; EONS, Early Onset Neonatal Sepsis; CI, Confidence Interval.

Studies Evaluating the Role of Interleukin 6 in Prediction of EONS

We included 2 studies assessing IL6. Two cut-offs were used: 8pg/mL[122] and 81pg/mL[61]. The individual unadjusted ORs of the 2 studies are shown in Figure 21. We did not pool ORs for these studies as they used different cut-offs.

Figure 21. Forest Plot Showing Individual Study Odds Ratios (Unadjusted) for Studies Evaluating IL6 at all cut-offs in prediction of EONS



IL6, Interleukin 6; MH, Mantel-Haenszel; EONS, Early Onset Neonatal Sepsis; CI, Confidence Interval.

Investigations for Heterogeneity and Sensitivity Analysis in the Prognostic Review

For the 4 studies evaluating CRP against the outcome of EONS at a cut-off of 10mg/L, the statistical heterogeneity was low (I^2 = 0%). There was however notable clinical heterogeneity in the measurement/definition of the outcome of interest, EONS. Studies differed with regard to the use of clinical and/or laboratory features in the definition and the duration of time after delivery that the diagnosis was made. No investigation for heterogeneity was carried out for studies evaluating PCT and IL6 as the studies were few and used different cut-offs.

No sensitivity analysis was carried out for the studies evaluating EONS as the number of studies in each group was less than 5. Studies assessing CRP were 5 in number but only 4 reported data with the same cut-off (10mg/L).

Characteristics of excluded studies

After full text review, 39 studies were excluded as they did not meet the inclusion criteria. Primary reasons for the exclusions are provided in Figure 2. Twenty two articles met the inclusion criteria but were excluded due to inability to extract 2x2 data for the patients with PPROM or due to unclear or conflicting 2x2 data. Compared to the included studies, the 22 studies were generally more recent in terms of publication dates. 16 of the 22 studies were published in the 15 years preceding the search date (2000 to 2015). 16 studies (73%) were prospective cohort designs, 5 retrospective cohort and 1 cross-sectional design. 20 studies assessed CRP, 6 assessed IL6 and 1 assessed PCT. 18 studies assessed HCA/Funisitis as the reference standard while 11 assessed EONS. Characteristics of these 22 studies are provided in Appendix 6.

Summary of Main Findings

In the diagnostic review, we included 14 studies reporting on 761 women with 361 episodes of HCA/Funisitis, a median prevalence of 41%. For studies evaluating CRP against the reference standard of HCA at a cut-off of 20mg/L, we obtained a pooled sensitivity of 59%, pooled specificity of 83%, LR+ of 3.45 and LR- of 0.50. We found a high level of heterogeneity which could be partially explained by the differences in cut-offs and interval from sampling to delivery. Sensitivity analyses show that the findings of the results are sensitive to patient selection criteria and the year of publication of the included studies. In general, the quality of the included studies was poor with majority judged to be at high risk of bias in at least 1 domain. Most studies were considered applicable to the review question with few having concerns for applicability with regard to patient selection. Findings of the diagnostic review are summarised in the Summary of Findings Table[116], Table 8.

In the prognostic review, we included 7 studies reporting data for 332 pregnancies with 97 episodes of EONS, a median prevalence of 26%. Our findings show that neonates born to mothers with PPROM and positive CRP (>=10mg/L) have 2.79 increased odds of having EONS compared to neonates born to mothers with PPROM with a negative CRP (<10mg/L). This is however, without adjusting for other confounders. Statistical heterogeneity in these studies was low though there was clinical heterogeneity with regards to the outcome characteristics. We did not pool the ORs for the studies evaluating PCT and IL6 as the index tests due to differences in cut-offs. No sensitivity analysis was carried out due to the small number of included studies. In general, the quality of included studies was poor with all studies judged to be at high risk of bias in at least one domain. There were also concerns for applicability particularly with the definition/ascertainment of the outcome. Findings of the prognostic review are summarised in the Summary of Findings Table,[116] Table 9.

Derivation of Additional Diagnostic Indices

Studies in the diagnostic review used different cut-offs for index tests. For studies using a cut-off of 20mg/L for CRP, we used the summary estimates obtained from HSROC analysis. This yielded values of sensitivity, specificity, LR+ and LR- with corresponding 95% CIs. For studies where several cut-offs were used, we obtained estimates of sensitivity from the SROC curves at a selected specificity[116] of 80% (false positive rate of 20%). The 20% false positive rate was selected as the minimum clinically acceptable false positive rate that could be reasonably obtained from the SROC plots. For studies evaluating CRP against HCA at all available cut-offs,

we obtained a sensitivity of 55% which corresponded to LR+ of 2.75 and LR– of 0.56. We were unable to obtain the sensitivity for studies evaluating PCT against HCA/Funisitis as the 80% specificity point on the curve was out of the range of values of the 2 included studies. For studies evaluating IL6 against HCA/Funisitis at all available cut-offs, we obtained a sensitivity of 62% which corresponded to an LR+ of 3.1 and LR– of 0.48. These additional indices were obtained from the corresponding curves and therefore do not have confidence intervals.[116]

Application of results to a hypothetical cohort (Normalised Frequencies)

To aid interpretation of the diagnostic review findings, we calculated normalised frequencies[116,134] with the following assumptions: A hypothetical cohort size of 100 patients with PPROM with a prevalence of histological chorioamnionitis of 40%, derived from the median prevalence of 41% from all included studies. For the prognostic review, we applied a hypothetical cohort size of 100 pregnant women and a prevalence of EONS of 25%, derived from the median prevalence of 26% from included studies. We rounded up the OR from 2.79 to 3, for ease of calculations. The impact of applying these tests is demonstrated in the Summary of Findings Tables, Table 8 and 9.

Table 8. Summary of Findings Table for the Diagnostic Review

Maternal Inflammatory Markers in the diagnosis of chorioamnionitis in preterm pre-labour rupture of membranes(PPROM), a systematic review							
Review Question	In pregnant women with PPROM, can maternal serum inflammatory markers be used to diagnose chorioamnionitis?						
Population	Pregnant women with PPROM						
Studies	Prospective cohort and Retrospective cohort studies from 1983 to 2014						
Index Test	C-reactive protein (CRP), Procalcitonin (PCT) and Interleukin 6(IL6) assessed in maternal serum before delivery						
Reference Standard	Histologic Chorioamnionitis and/ or funisitis						
Prevalence of disease	Median 41% (Range 21% - 63%, IQR 36% to 53%) 761 women with 361 episodes of HCA/Funisitis						
Quality	Included Studies were generally of poor quality with all studies at high risk of bias in at least one domain (QUADAS-2)						
Index Test	Studies (Participants)	Sensitivity	Specificity	Likelihood Ratio Positive	Likelihood Ratio Negative	Heterogeneity	Interpretation Assuming a prevalence of HCA of 40%*, testing 100 pregnant women will yield the following results
CRP at 20mg/L†	5 (252)	59% (48-69)	83% (74-89)	3.45 (2.24-5.30)	0.50 (0.38-0.64)	?Moderate Sources not assessed due to small number of studies	Of the 40 with disease, the test will correctly diagnose 24, 16 will be missed. Of the 60 without disease, the test will correctly detect 50, 10 will be wrongly diagnosed as having disease.
CRP at all cut-offs‡	11 (570)	55%	80%	2.75	0.56	High Likely sources: interval of sampling time to delivery, assay type	Of the 40 with disease, the test will correctly diagnose 22, 18 will be missed. Of the 60 without disease, the test will correctly detect 48, 12 will be wrongly diagnosed as having disease.
PCT at all cut-offs§	2 (80)	-	-	-	-	-	-
IL6 at all‡ cut-offs	3 (191)	62%	80%	3.1	0.48	Sources not assessed due to small number of studies	Of the 40 with disease, the test will correctly diagnose 25, 15 will be missed. Of the 60 without disease, the test will correctly detect 48, 12 will be wrongly diagnosed as having disease.

**Prevalence of disease selected from median prevalence in included studies. †Results from HSROC meta-analysis. ‡Derived from SROC curves assuming a specificity of 80% (False positive rate of 20%). ‡Unable to determine measures from the SROC curve at a FP rate of 20% (Available range of results do not encompass this FP rate)*

Table 9. Summary of Findings Table for the Prognostic Review

Maternal Inflammatory Markers in the prediction of Early Onset Neonatal Sepsis in preterm pre-labour rupture of membranes(PPROM), a systematic review				
Review Question	In pregnant women with PPROM, can maternal serum inflammatory markers be used to predict early onset neonatal sepsis (EONS)?			
Population	Pregnant women with PPROM			
Studies	Prospective cohort and Retrospective cohort studies from 2005 to 2014			
Index Test	C-reactive protein (CRP), Procalcitonin (PCT) and Interleukin 6(IL6) assessed in maternal serum before delivery			
Reference Standard	Early Onset Neonatal Sepsis This definition includes features of infection or sepsis (clinical and/or laboratory) diagnosed at any time in the first week of life or where neonatal infection or sepsis is designated 'early'			
Prevalence of outcome	Median prevalence 26% (range 19% to 44%, IQR 26-34%) 97 episodes of EONS in 332 pregnancies			
Quality	Included Studies were generally of poor quality with all studies at high risk of bias in at least one domain (QUIPS)			
Index Test	Studies (Participants)	Odds Ratio (95% CI)	Heterogeneity	Interpretation Assuming a prevalence of 25%*, testing 100 pregnant women will yield the following results
CRP at 10mg/L[†]	4 (161)	2.79 (1.33 – 5.88), p = 0.007	Chi^2 p=0.49, I^2 = 0%, Very low	(OR assumed to be 3) 40 mothers will test positive, 15 of their babies will have EONS (38%). 60 mothers will test negative, 10 of their babies will have EONS (17%). Of the 25 babies with EONS, 15 will have been predicted by the maternal test (60%).
CRP at 20mg/L[‡]	1 (73)	5.09 (0.91-28.60)	Not assessed	
PCT at all cut-offs[§]	2 (80)	0.93(0.28-3.03) – 3.13 (0.71-13.81)	Not assessed	
IL6 at all cut-offs[§]	2 (98)	39.48(2.13-731.15) – 333.00(28.29-3919.78)	Not assessed	

QUIPS, Quality in Prognostic Studies. *Prevalence of Disease selected from the median prevalence in included studies. [†]Pooled OR, random effects model. [‡]No pooling. Only 1 study available at this cut-off. [§]No pooling. Available studies report results at different cut-offs.

DISCUSSION

We undertook to assess whether inflammatory markers CRP, PCT and IL6 can be useful in the management of PPROM by aiding in diagnosis of HCA and/or funisitis and whether these tests can further predict which neonates will develop EONS. The results of the diagnostic review show high false positive rates (low specificity) and high false negative rates (low sensitivity). The corresponding likelihood ratios (both positive and negative) show only small changes in probability of or absence of disease. The prognostic review shows slightly increased odds of disease in neonates born to mothers with a positive CRP. These findings are obtained in the background of few included studies with generally small sample sizes, poor quality assessments and significant heterogeneity.

Comparison of Findings with previous and related reviews

There are a number of similar systematic reviews that have been published examining inflammatory markers and their ability to diagnose chorioamnionitis and predict neonatal sepsis [17,82,83](Table 1). Trochez-Martinez *et al*[82] and Van de Laar *et al*[83] both assessed the role of CRP in predicting chorioamnionitis in the context of PPROM. Both reviews had few studies, high between study heterogeneity and differences in cut-offs that limited their ability to do pooled analysis. Through our broader search criteria, our review identified more studies than both these 2 reviews. We also demonstrated high heterogeneity but unlike these reviews we were able to use recommended meta-analytic methods that allowed pooling despite differences in cut-offs.[109] We also characterised the heterogeneity and identified some of its likely sources. Despite these differences, our findings are in agreement that there is not clear evidence to support use of CRP in the diagnosis of chorioamnionitis.

A more extensive and more recent review was conducted by Su *et al.*[17] This review assessed multiple markers including CRP, PCT and IL6 evaluated them against the outcome of EONS. However, this review was not limited to the clinical condition of PPROM as it included pregnancies of any gestation and a variety of clinical conditions in pregnancy. Because of this, the review identified more studies than ours, 8 studies for CRP-EONS (compared to 5 in our review) and 5 studies for IL6-EONS (compared to 2 in our review). Our findings are therefore not directly comparable to this review. That review pooled analysis from the different studies regardless of differences in cut-offs and it is not clear what the summary estimates obtained in this review refer to. The review concluded that only IL6 was found to be sufficient to rule in EONS, CRP and PCT showing no useful role.

Qualifying the Evidence

The findings of this review need to be evaluated with the knowledge of various strengths and weaknesses both from the included studies as well as those of the review methods.

Strengths and weaknesses of Included Studies

Studies included into the review were few in number and generally had small sample sizes. This affects the precision and applicability of the findings, especially in the face of substantial heterogeneity. Specifically, there were very few studies assessing PCT and IL6 in maternal serum. Further, included studies reported diagnostic performance of the tests at different cut-offs limiting the number of studies available for obtaining summary estimates in the diagnostic review and for pooling in the prognostic review.

Included studies were found to be of poor quality with all studies at high risk of bias in 1 or more domains. Poor reporting in primary studies limited the assessment of methodological quality and applicability of the included studies. Because of this, our study findings may be strongly affected by different biases.[135]

A selection bias may exist due to the inappropriate selection of patients for inclusion into the individual studies. Choosing patients less likely to have disease, such as patients who have longer latency periods after PPROM,[121,123] may result in lower false negative rates.[135] Choosing patients more likely to have disease, such as patients with clinical features of infection, may result in fewer false positives. Rather than use pre-specified cut-offs, several studies used cut-offs derived from the study data. This tends to select cut-offs with optimal characteristics of specificity and sensitivity and overestimates the diagnostic accuracy of the test.[136] Incorporation bias arising from a lack of blinding of outcome assessors may also overestimate diagnostic accuracy[135] by causing intentional or subconscious alteration of the results of the reference standard or outcome. A lack of blinding of the caregivers may also alter subsequent management of patients with PPROM and in turn affect the results. Elevated levels of index test may lead to immediate intervention and delivery which would in turn reduce the risk of HCA/Funisitis. Provision of prophylactic antibiotics based on index test results may also reduce the risk of EONS. A long interval between the index test and the assessment of the reference standard may result in a misclassification bias as the disease state may change during the interval. Evidence of this was demonstrated in the investigation of heterogeneity in CRP-

HCA/Funisitis studies where studies with a shorter interval reported better diagnostic accuracy than studies with a long interval (Figure 12b).

Concerns for applicability to the review question were few in the diagnostic review with most included studies closely matching the predefined criteria. In the prognostic review, there were concerns for applicability in the definition and ascertainment of the outcome, EONS. Some of the definitions of the outcome of interest did not closely match the predefined criteria. This could have influenced the findings of the review.

Strengths and weaknesses of the review process

We have conducted this review following guidelines and methods recommended by the Cochrane group of diagnostic reviews[109] and the Cochrane prognosis review methods.[91] The review followed a registered protocol.[94] Criteria for eligibility of studies was determined beforehand and adhered to throughout the selection processes. We set out to study the performance of specific diagnostic tests in a specific sample (maternal blood/serum) in a specific clinical condition, PPROM. Limiting the review to a specific clinical condition in pregnancy would reduce chances of pooling together test accuracy indices that are different due to differences in patient characteristics and probability of disease.[135] HCA/Funisitis was chosen as the reference standard for the diagnostic review due to the objectivity of its assessment[78] and its correlation with infectious complications in the mother and baby.[63,80]

Several steps of the review process were undertaken by two independent reviewers with consensus employed whenever conflict arose. The high level of agreement between the reviewers in steps determining inclusion of studies into the review reduces the probability that appropriate studies were excluded from the review or that inappropriate studies were excluded.[116]

Another strength of this review lies in the comprehensive electronic search in 3 databases supported by a search of reference lists of included studies and previous related reviews. We employed a broad search strategy with search terms that did not include the outcomes or reference standards.[98] No filter for 'diagnostic studies' was used as this would have excluded eligible studies that were not explicitly labelled as diagnostic studies.[98] This search strategy enabled us to identify a larger number of articles for initial screening and a larger number of potentially eligible studies compared to previous systematic reviews.[17,82,83]

However, a large proportion of potentially eligible studies were excluded due to inability to extract 2x2 data. Despite contacting authors of these studies, no additional data were obtained. We have outlined characteristics of these excluded studies and the differences between them and the included studies. While the impact of these excluded studies could not be assessed directly, it is likely that the results of the review would be altered if their data were available.

Further, we were unable to translate non English articles. This could have affected the number of included studies, and the review findings, if the non-English articles would have been eligible for inclusion into the review. In addition, we were unable to obtain full texts of 3 articles despite extensive search and inter-library networking. Inability to translate and retrieve these articles could have introduced a reporting bias, the magnitude of which we are unable to assess. Another limitation is in limiting the review to published studies only, a feature that limits the representability of the review. This could also introduce bias if unpublished studies or studies available from other sources demonstrated different diagnostic performances from published studies.

Analysis methods employed in this review follow recommendations from the Cochrane group[108]. This is in contrast to previous related reviews which have used meta-analytic methods now known to be flawed.[17,82,87] Use of several cut-offs limited pooling of diagnostic indices. We overcame this limitation in the diagnostic review by using meta-analytic methods[109,107] that allow for pooling of studies with different cut-offs hence making efficient use of the available data and maximising power.[109] Where the number of studies allowed, we carried out assessments for heterogeneity. Subgroups created for this purpose were determined *a priori* and were based on reasonable assumptions. We carried out these assessments by meta-regression but assessed each characteristic in turn. Multivariable analysis including several subgroup characteristics into the model at the same time was not carried out as this would be affected by the low power in the setting of few studies.[109] For the same reason, we simplified the models by assuming the shape parameter in the different subgroups to be the same.[109]

The review did not limit studies by year of publication and included studies spanned a period of many years. Sensitivity analysis on CRP-HCA/Funisitis studies demonstrated poorer diagnostic performance when the studies were limited to those published in the preceding 15 years (2000 to 2015). This could have arisen from differences in performance of the index test, methods of assessment of the reference standard or differences in publication of studies in these time

periods. Heterogeneity assessment showed that diagnostic performance differed in the periods before and after CRP standardisation and this may partially explain this finding. Another plausible explanation is a publication bias. Older studies may have had selective publication favouring only positive or significant results with recent studies being more likely to report all findings regardless of the result. It may also be related to poorer study methodologies and weaker research governance and monitoring that may have existed in that time and leading us to question the validity of these older studies.

Applicability of findings to review question

In the diagnostic review, all included studies had low concerns for applicability in the index test and reference standard domains. This was due to strict adherence to inclusion criteria for eligibility of studies to the review. However, high applicability concerns arose in the patient selection domain particularly due to failure to explicitly exclude patients with preterm labour in the included studies. This judgement could also have been affected by poor reporting of inclusion and exclusion criteria in the studies. Patients with preterm labour are likely to differ in their infection risk and in performance of diagnostic tests.[137] Another concern was with the assessment of gestational age where many studies did not report any ultrasound confirmations of gestational age. Though assessment of gestational age was not included formally into the applicability assessments, it is an important factor in interpreting the findings of this review as the role of the tests may vary with gestational age.[13]

In the prognostic review, there were concerns in the definition of the outcome of EONS with regard to duration after delivery and use of laboratory and/or clinical features in establishing the diagnosis. The ideal definition would use a combination of laboratory and clinical features and specify duration of time after delivery, in this case, within the first 1 week.[81,96] The poor applicability of studies with regards to the outcome of interest should be noted when interpreting the findings of this review.

Conclusions

Implications for Clinical Practice

The proposed clinical role of the tests in the setting of PPROM is to guide interventions by appropriately identifying which pregnancies have infection. PPROM in the absence of infection is generally managed expectantly[1]. Once infection is diagnosed or suspected, the management changes to administration of parenteral antibiotics and interventions for delivery. Both management options have important consequences. Interventions for delivery result in the birth

of a preterm baby and attendant complications of prematurity. Delaying delivery in the presence of infection results in a higher risk of maternal systemic infection and transmission of infection to the foetus with eventual birth of a baby with neonatal infection and related complications. Prognosis for babies born preterm with infectious morbidity is poorer than for preterm babies of similar gestational age with no infection.[8,9]

A false positive test result would result in an iatrogenic preterm birth in a pregnancy that would have been safely prolonged while a false negative result would delay interventions and lead to more infection related complications. False negatives have other opportunities for detection of infection from further laboratory or clinical tests. Because delivery is irreversible, the negative implications of a false positive test are greater. The impact of the test is also dependent on gestational age. False positive tests in shorter gestations have greater impact due to greater concern for neonatal outcome and survival. False negatives have greater impact for longer gestations as infection here would alter outcomes in a neonate with otherwise good prognosis.

There is insufficient evidence to recommend use of CRP, PCT or IL6 in maternal blood for the diagnosis of HCA/Funisitis in PPROM. The slightly increased odds of EONS in mothers with CRP>10mg/L is not large enough to inform interventions such as delivery. It may, however, justify closer follow-up and investigations for the new-borns and perhaps a lower threshold for initiation of antibiotics.

Whether use of these tests should be recommended depends on existence of and the diagnostic performance of alternative tests in similar roles. For mothers, samples such as amniotic fluid may offer an alternate approach. Tests in amniotic fluid appear to have better diagnostic performance than tests in maternal serum[12] but are limited by the complexity of amniotic fluid collection, increased costs and lower acceptability to women. Another sample that can be analysed is cord blood collected at delivery. The sample is easy to obtain and may better predict neonatal infection.[17] Nonetheless, maternal blood still offers the advantage of being available before delivery and hence able to inform decision making during latency. An alternative approach would be to combine tests in maternal serum with other laboratory and clinical markers. The performance of these tests may improve if included in a model with other factors.

Implications for Research

This review has demonstrated several weaknesses in the included studies and significant heterogeneity in the findings of the review that limit our ability to make reliable. There is need for a better designed study to reliably answer the review's question.

We recommend a prospective cohort design with consecutive recruitment of mothers diagnosed with PPROM who are eligible for expectant management. The diagnosis of ROM should be made by reliable clinical examination with confirmatory tests applied in less certain cases. Preterm labour should be excluded and management should follow current guidelines.[1] Gestational age should be confirmed in all pregnancies by reliable dating ultrasound earlier in the pregnancy. We recommend serial sampling of maternal blood so as to ensure an appropriate interval between sampling and delivery is maintained. Standardised assessment and documentation of clinical features should be done regularly. Reliable methods should be used in the assay of the index test. Standard protocols for handling and assessing the placenta should be put in place and a standardised and current definition of HCA and Funisitis employed. The outcome assessors/pathologists should be blinded to the results of the index test. After delivery, all new-borns should undergo standardised clinical and laboratory evaluation and outcome assessed using standard definitions. Where possible, outcome assessors should be blinded to results of the index test. In addition to the outcome of EONS, other outcomes that could be assessed include admission to neonatal intensive care and neonatal mortality. The analytic methods should rely on a predetermined cut-off. Since a universal cut-off has not been agreed on for this condition, using several cut-offs is recommended. The analysis should also account for potential confounders and/or independent risk factors such as gestational age, antibiotic use and latency period. In addition to assessing the role of the inflammatory marker, other clinical and laboratory factors should be assessed jointly by logistic regression and construction of a prediction model.

Several studies included in this report were poorly reported. This made quality assessments and data extraction difficult. We recommend that diagnostic accuracy studies be reported following the recommended Standards for Reporting of Diagnostic Accuracy – STARD.[138] This will enable reviewers to correctly assess these studies and will make more data available for review.

STATEMENT OF CONJOINT WORK

The first reviewer, Angela Koech Etyang (AKE) played the primary role in the development of the proposal, the review processes and production of the report. These roles were carried out under the guidance of the supervisors. The electronic search strategy was prepared by AKE and reviewed by the University Librarian, Nasra Gathoni.

The supervisors, Mwaniki Mukaindo (MM), Geoffrey Omuse (GO) and Marleen Temmerman (MT) also played additional roles in the review processes that required more than one reviewer: screening articles for inclusion, data extraction and quality assessment. Specific roles played are outlined in Table 10.

Table 10. Roles of Reviewers

	Reviewer	Electronic Search	Screening articles for Inclusion	Data Extraction	Quality Assessment	Data Analysis	Graph Production	Report Writing
1	AKE	√	√	√	√	√	√	√
2	MM		√	√	√			
3	GO		√	√	√			
2	MT		√*		√*			

*Resolving Conflicts in case of disagreements between other reviewers.

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APPENDICES

Appendix 1 – Electronic Databases Search Strategy

Appendix 1a. Search Strategy for Medline

Table 1a: Ovid Medline® search strategy

Provider/Interface Ovid
Database Medline®
Date searched 29th October 2015
Database update
Search developer(s) Koech
Limit to English? No
Date Range 1946 to October, week 4 2015 (from Inception to Search Date)

1	exp Fetal Membranes, Premature Rupture/
2	rupture of membranes.af.
3	drainage of liquor.af.
4	amniorrhexis.af.
5	amniorrhea.af.
6	fetal membrane* .af.
7	foetal membrane* .af.
8	amniorrhoea.af.
9	amniorhexis.af.
10	amniotic sac.af.
11	amniotic fluid.af.
12	exp C-Reactive Protein/
13	c reactive protein.af.
14	*crp.af.
15	procalcitonin.af.
16	pct.af.
17	exp Interleukin-6/
18	interleukin 6.af.
19	il6.af.
20	il-6.af.
21	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11
22	12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20
23	21 and 22
24	limit 23 to humans

1

Appendix 1b. Search Strategy for EMBASE

Table 1b: Ovid EMBASE® search strategy

Provider/Interface	Ovid
Database	EMBASE
Date searched	29th October 2015
Database update	
Search developer(s)	Koech
English only?	No
Date Range	1947 to 28 October 2015 (From Inception to Search Date)

1	exp Fetal Membranes, Premature Rupture/
2	rupture of membranes.af.
3	drainage of liquor.af.
4	amniorrhexis.af.
5	amniorrhea.af.
6	fetal membrane* .af.
7	foetal membrane* .af.
8	amniorrhoea.af.
9	amniorhexis.af.
10	amniotic sac.af.
11	amniotic fluid.af.
12	exp C-Reactive Protein/
13	c reactive protein.af.
14	*crp.af.
15	procalcitonin.af.
16	pct.af.
17	exp Interleukin-6/
18	interleukin 6.af.
19	il6.af.
20	il-6.af.
21	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11
22	12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20
23	21 and 22
24	limit 23 to humans

2

Appendix 1c. Search Strategy for The Cochrane Library

Table 1c: PubMed search strategy

Provider/Interface	Wiley
Database	The Cochrane Library
Date searched	29 October 2015
Database update	
Search developer(s)	Koech
English only?	No
Date Range	From Inception to Search Date

#1	MeSH descriptor: [Fetal Membranes, Premature Rupture] explode all trees384
#2	rupture of membranes (Word variations have been searched)789
#3	drainage of liquor (Word variations have been searched)1
#4	amniorrhexis (Word variations have been searched)2
#5	amniorrhea (Word variations have been searched)0
#6	fetal membrane* (Word variations have been searched)400
#7	foetal membrane* (Word variations have been searched)11
#8	amniorrhoea (Word variations have been searched)0
#9	amniorhexis (Word variations have been searched)0
#10	amniotic sac (Word variations have been searched)22
#11	amniotic fluid (Word variations have been searched)680
#12	MeSH descriptor: [C-Reactive Protein] explode all trees3117
#13	c reactive protein (Word variations have been searched)8428
#14	*crp (Word variations have been searched)5340
#15	procalcitonin (Word variations have been searched)382
#16	pct (Word variations have been searched)457
#17	MeSH descriptor: [Interleukin-6] explode all trees2127
#18	interleukin 6 (Word variations have been searched)8204
#19	IL6 (Word variations have been searched)212
#20	IL-6 (Word variations have been searched)4380
#21	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11
#22	#12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR
#23	#21 AND #22

3

Appendix 2. Data Extraction Form

Study ID _____

Inflammatory Markers for PPROM, Systematic Review, Data Extraction Form

Name of Reviewer Completing the Form _____

Date form completed_____

Study ID (Author/Year) _____ /_____**Citation ID(s)** _____

Section 1. Study Characteristics

Study Design

◊ Prospective Cohort ◊ Retrospective cohort ◊ Case control

◊ Case control ◊ Cross-sectional study ◊ Clinical Trial

◊ Other_____

Study Setting; Country _____

Health Facility _____

Study Period; From (Month/Year) _____to _____

Method of Recruitment of Participants/ Method of Sampling

◊ Prospective ◊ Retrospective ◊ Other_____

◊ Consecutive ◊ Random sampling ◊ Not Stated

Other Information _____

_____

Participants and Patient Flow

Number of Participants/ Sample size_____ (Note: Include numbers with clinical condition of interest - PPROM only)

Gestational Age (GA) Range ___-___weeks

Method of ascertaining GA _____

Diagnosis of PPROM _____

Confirmation of PPROM_____

Inclusion Criteria

_____ _____ _____

_____ _____ _____

_____ _____ _____

Exclusion Criteria

_____ _____ _____

_____ _____ _____

Routine Management of PPROM, Including monitoring

◊ Antibiotics Criteria _____
◊ Steroids Criteria _____
◊ Tocolytics Criteria _____
◊Other_____ _____ _____ _____

1

Did the clinicians managing the patients know the results of the index test?

◊Yes ◊No ◊ Unclear

Did the clinicians use the results of the index test in the management of the patients?

◊Yes ◊No ◊ Unclear

Were any patients excluded after initially meeting the inclusion criteria?

◊Yes ◊No ◊ Unclear

If Yes, How many were excluded? _____

Give details/ reasons for exclusion_____

_____

(Optional)

Sketch the study flow diagram for the patients with the clinical condition of interest

Study ID _____

Section 2a. Index Test

(Multiple pages for section 2 should be used if more than one marker is assessed in the same study)

◊ CRP ◊ PCT ◊ IL6

Site of Assay ◊ Laboratory ◊Bedside

Method of Assay_____

Name of assay/ Manufacturer _____

Units of Assay_____Lowest detection limit of assay/Sensitivity _____

Cut-off(s) for positive test _____

◊Predetermined cut-off ◊Determined from ROC analysis

◊Other_____

Timing of test relative to time of PPROM _____

Timing of test relative to time of admission _____

Timing of test relative to delivery _____

_____

Other relevant information _____

_____

_____

Section 2b. Index Test

(Multiple pages for section 2 should be used if more than one marker is assessed in the same study)

◊ CRP ◊ PCT ◊ IL6

Site of Assay ◊ Laboratory ◊Bedside

Method of Assay_____

Name of assay/ Manufacturer _____

Units of Assay_____Lowest detection limit of assay/Sensitivity _____

Cut-off(s) for positive test _____

◊Predetermined cut-off ◊Determined from ROC analysis

◊Other_____

Timing of test relative to time of PPROM _____

Timing of test relative to time of admission _____

Timing of test relative to delivery _____

_____

Other relevant information _____

_____

_____

Study ID _____

Section 3a. Reference Standard: Histologic Chorioamnionitis (HCA)

Reference Standard (tick all that apply):

◊Histologic Chorioamnionitis ◊Funisitis ◊Other _____

Diagnostic Criteria

Is a diagnostic criteria for the reference standard specified? ◊Yes ◊No

Is a reference to a source of diagnostic criteria given? ◊Yes ◊No

Assessment of the reference standard:

Who performed the placental assessment? _____

Were the assessors blinded to outcome of index test? ◊Yes ◊No ◊ Unclear

Were all placentas assessed the same way? ◊Yes ◊No ◊ Unclear

Number of patients who had the reference standard (HCA) assessed _____

Are there any placentas / patients whose outcome is not reported? _____

Is there a reason given for this?_____ _____

_____

Section 3b. Reference Standard: Early Onset Neonatal Sepsis (EONS)

Diagnostic Criteria

Is a diagnostic criteria for the reference standard specified? ◊Yes ◊No

Is a reference to a source of diagnostic criteria given? ◊Yes ◊No

What is the duration of time for ascertainment of EONS? _____hrs

Does the criteria include: ◊Confirmed EONS ◊Suspected EONS ◊ Both

Assessment of new-borns for EONS:

Who ascertained the outcome of EONS in the new-borns? _____

Were the assessors blinded to outcome of index test? ◊Yes ◊No ◊ Unclear

Were all outcome assessments done the same way for all new-borns? ◊Yes ◊No ◊ Unclear

Number of babies who were assessed for EONS_____

Are there any babies whose outcome is not reported? _____

Is there a reason given for this?_____ _____

_____

Study ID _____

Section 4a. Statistical Measures of Diagnostic Accuracy

(Multiple pages for section 4 should be used if more than one marker is assessed in the same study)

Number of patients with an outcome of HCA _____

Number of patients with a positive test on the index test _____

		Reference Standard: HCA		
		Present	Absent	Total
Index Test: _____	Positive (> cutoff)			
	Negative (< cutoff)			
	Total			

Sensitivity _____%

Specificity _____%

Positive Predictive Value _____%

Negative Predictive Value _____%

Likelihood Ratio Positive_____

Likelihood Ratio Negative_____

Diagnostic Odds Ratio _____

Area Under the Curve_____

Pearson's Correlation_____

Others_____

_____

_____

Section 4b. Statistical Measures of Diagnostic Accuracy

(Multiple pages for section 4 should be used if more than one marker is assessed in the same study)

Number of patients with an outcome of HCA _____

Number of patients with a positive test on the index test _____

		Reference Standard: HCA		
		Present	Absent	Total
Index Test: _____	Positive (> cutoff)			
	Negative (< cutoff)			
	Total			

Sensitivity _____%

Specificity _____%

Positive Predictive Value _____%

Negative Predictive Value _____%

Likelihood Ratio Positive_____

Likelihood Ratio Negative_____

Diagnostic Odds Ratio _____

Area Under the Curve_____

Pearson's Correlation_____

Others_____

_____

_____

Study ID _____

Section 5a. Statistical Measures of Prognosis

(Multiple pages for section 5 should be used if more than one marker is assessed in the same study)

Number of patients with an outcome of EONS _____

Number of patients with a positive test on the index test _____

		Reference Standard: EONS		
		Present	Absent	Total
Index Test: _____	Positive (> cutoff)			
	Negative (< cutoff)			
	Total			

Odds Ratio (and 95% CI) _____ Relative Risk (and 95% CI) _____

Hazard Ratio (and 95% CI) _____

Sensitivity _____% Specificity _____%

Positive Predictive Value _____% Negative Predictive Value _____%

Likelihood Ratio Positive_____ Likelihood Ratio Negative_____

Diagnostic Odds Ratio _____ Area Under the Curve_____

Pearson's Correlation_____

Others_____

_____

Section 5b. Statistical Measures of Prognosis

(Multiple pages for section 5 should be used if more than one marker is assessed in the same study)

Number of patients with an outcome of EONS _____

Number of patients with a positive test on the index test _____

		Reference Standard: EONS		
		Present	Absent	Total
Index Test: _____	Positive (> cutoff)			
	Negative (< cutoff)			
	Total			

Odds Ratio (and 95% CI) _____ Relative Risk (and 95% CI) _____

Hazard Ratio (and 95% CI) _____

Sensitivity _____% Specificity _____%

Positive Predictive Value _____% Negative Predictive Value _____%

Likelihood Ratio Positive_____ Likelihood Ratio Negative_____

Diagnostic Odds Ratio _____ Area Under the Curve_____

Pearson's Correlation_____

Others_____

Study ID _____

Section 6. Contact Authors

Do the authors need to be contacted for more data? ◊Yes ◊No

Outline the data items to be requested from the authors.

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____

Notes/Comments

_____

Appendix 3a. QUADAS Tool (Data Extraction Form)

Study ID _____

Inflammatory Markers for PPROM, Systematic Review, QUADAS – 2 Tool

Name of Reviewer Completing the Form _____

Date form completed_____

Study ID (Author/Year) _____/_____ Citation ID(s) _____

QUADAS-2 tool: Risk of bias and applicability judgments

Domain 1: Patient selection			
A. Risk of bias			
• Was a consecutive or random sample of patients enrolled?	◊ Yes	◊ No	◊ Unclear
• Was a case-control design avoided?	◊ Yes	◊ No	◊ Unclear
• Did the study avoid inappropriate exclusions?	◊ Yes	◊ No	◊ Unclear
Could the selection of patients have introduced bias?	RISK: LOW/HIGH/UNCLEAR		
B. Concerns regarding applicability			
Is there concern that the included patients do not match the review question?	CONCERN: LOW/HIGH/UNCLEAR		
Domain 2: Index test(s) _____ *(if more than 1 index test was used, please complete for each test)*			
A. Risk of bias			
• Were the index test results interpreted without knowledge of the results of the reference standard?	◊ Yes	◊ No	◊ Unclear
• If a threshold was used, was it pre-specified?	◊ Yes	◊ No	◊ Unclear
Could the conduct or interpretation of the index test have introduced bias?	RISK: LOW/HIGH/UNCLEAR		
B. Concerns regarding applicability			
Is there concern that the index test, its conduct, or interpretation differ from the review question?	CONCERN: LOW/HIGH/UNCLEAR		
Domain 2: Index test(s) _____ *(if more than 1 index test was used, please complete for each test)*			
C. Risk of bias			
• Were the index test results interpreted without knowledge of the results of the reference standard?	◊ Yes	◊ No	◊ Unclear
• If a threshold was used, was it pre-specified?	◊ Yes	◊ No	◊ Unclear
Could the conduct or interpretation of the index test have introduced bias?	RISK: LOW/HIGH/UNCLEAR		
D. Concerns regarding applicability			
Is there concern that the index test, its conduct, or interpretation differ from the review question?	CONCERN: LOW/HIGH/UNCLEAR		

1

Domain 2: Index test(s) _____ *(if more than 1 index test was used, please complete for each test)*			
E. Risk of bias			
• Were the index test results interpreted without knowledge of the results of the reference standard?	◊ Yes	◊ No	◊ Unclear
• If a threshold was used, was it pre-specified?	◊ Yes	◊ No	◊ Unclear
Could the conduct or interpretation of the index test have introduced bias?	RISK: LOW/HIGH/UNCLEAR		
F. Concerns regarding applicability			
Is there concern that the index test, its conduct, or interpretation differ from the review question?	CONCERN: LOW/HIGH/UNCLEAR		

Domain 3: Reference standard			
A. Risk of bias			
• Is the reference standard likely to correctly classify the target condition?	◊ Yes	◊ No	◊ Unclear
• Were the reference standard results interpreted without knowledge of the results of the index test?	◊ Yes	◊ No	◊ Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	RISK: LOW/HIGH/UNCLEAR		
B. Concerns regarding applicability			
Is there concern that the target condition (HCA) as defined by the reference standard does not match the review question?	CONCERN: LOW/HIGH/UNCLEAR		

Domain 4: Flow and timing			
A. Risk of bias			
• Was there an appropriate interval between index test(s) and reference standard?	◊ Yes	◊ No	◊ Unclear
• Did all patients receive the same reference standard?	◊ Yes	◊ No	◊ Unclear
• Were at least 90% of eligible patients included in the analysis?	◊ Yes	◊ No	◊ Unclear
Could the patient flow have introduced bias?	RISK: LOW/HIGH/UNCLEAR		

Appendix 3b. QUADAS Rating Guidance Tool

Quality in Diagnostic Accuracy Studies (QUADAS) – 2, Rating Guidance Tool
The review question:
In pregnant women with preterm pre-labour rupture of membranes (PPROM), can maternal serum inflammatory markers (the index test) be used to **diagnose** patients with chorioamnionitis?
Index test: CRP, PCT or IL6 in maternal blood / serum.
Reference standard: Histologic Chorioamnionitis (HCA)
Setting: Any clinical setting.
Patients: Patients with PPROM <37 weeks gestation. (*Ideally, the study should exclude patients with preterm labour).*
Intended use: The test should be used before delivery, after development of PPROM. *(Ideally before development of clinical features of chorioamnionitis).*
Rating Guidance for QUADAS -2

Domain 1: Patient selection
A. Risk of bias
• **Was a consecutive or random sample of patients enrolled?** To score a 'Yes' a study had to have specified that a random or consecutive sample was enrolled. Studies stating that 'All' patients with the condition were enrolled or offered enrollment will be assumed to be consecutively sampled.
• **Was a case-control design avoided?** Any study design other than a case control design is scored 'Yes'.
• **Did the study avoid inappropriate exclusions?** Studies should exclude women with clinical features of chorioamnionitis at the time of admission as these women do not present a diagnostic or management dilemma. Studies whould not limit women to specific time durations after PPROM. Studies should not select women based on availability or ability to perform other tests. Studies should not exclude women with other pregnancy related or medical conditions that commonly coexist with PPROM. Exclusions for inflammatory conditions that are known to cause a rise in inflammatory markers was acceptable. Exclusion of extrauterine infections was acceptable as these are known to cause a rise in inflammatory markers. Studies should not select women based on availability of test results or complete records.
Could the selection of patients have introduced bias? If a study scores a 'No' in any of the above, it should be rated 'High Risk' of Bias.
B. Concerns regarding applicability
Is there concern that the included patients do not match the review question? The target population is women with preterm PPROM. The study should show a clear diagnosis of PPROM in all included women. The study should mention exclusion of women in preterm labour. For this an explicit mention of exclusion based on cervical diation was ideal. Exclusion of contractions was accepted.
Domain 2: Index test(s)
A. Risk of bias
• **Were the index test results interpreted without knowledge of the results of the reference standard?** 'No' if the index test was assessed before delivery. 'No' if the method of assessment is objective, say if it is an automated assay.

1

- **If a threshold was used, was it pre-specified?**

  For a study to score a Yes it should be clearly stated that the cutoff was predetermined. Cut-offs derived from the data eg. From ROC curves, or optimising for sensitivity or specificity should score a 'No'.

Could the conduct or interpretation of the index test have introduced bias?

A 'No' in the above question should be scored as a High Risk of Bias

B. Concerns regarding applicability

Is there concern that the index test, its conduct, or interpretation differ from the review question?

The test should be described in sufficient detail.

Domain 3: Reference standard

A. Risk of bias

- **Is the reference standard likely to correctly classify the target condition?**

  This refers to whether a standard and referenced definition or diagnostic criteria for HCA has been used. If a clear and objective definition has been used then this should score a Yes. If a vague description with no reference to a standard document is given this should score a No. Ideally HCA should be assessed by a pathologist.

- **Were the reference standard results interpreted without knowledge of the results of the index test?**

  Studies with no blinding should score a 'No'.

Could the reference standard, its conduct, or its interpretation have introduced bias?

A study scoring a 'No' in any of the above two questions should be rated 'High Risk' of bias. A study scoring 'unclear' with reference to blinding will be considered 'high risk.'

B. Concerns regarding applicability

Is there concern that the target condition (HCA) as defined by the reference standard does not match the review question?

The review is limited to studies using Histologic Chorioamnionitis and funisitis as the reference standard

Domain 4: Flow and timing

A. Risk of bias

- **Was there an appropriate interval between index test(s) and reference standard?**

  We shall consider an appropriate interval to be one that is nearer delivery. An interval of 72 hours before delivery will be considered appropriate.

- **Did all patients receive the same reference standard?**

  Did all patients receive confirmation of the diagnosis by the same reference standard or did somepatients receive a different reference standard? If yes, then score a 'Yes'.

- **Were at least 90% of eligible patients included in the analysis?**

  This refers to missing data / patients loss to follow up. Ie patients who already met the inclusion criteria but were then excluded from the analysis for various reasons, eg. Missing data, or unavailable placenta or no reasons given. Or if additional criteria were used for including women in the analysis, eg, specific sampling times.

  All patients included in the study (or at least 90% of them) should be included / reported in the 2x2 table of results.

Could the patient flow have introduced bias?

A study scoring a No in any of the above two questions should be rated 'High Risk' of bias.

Appendix 4a. QUIPS tool (Data Extraction Form)

Study ID _____

Inflammatory Markers for PPROM, Systematic Review, QUIPS Tool

Name of Reviewer Completing the Form _____

Date form completed_____

Study ID (Author/Year) _____/_____ **Citation ID(s)** _____

Key

Y – Yes, N – No, U- Unclear, n/a – Not applicable

QUIPS tool: Risk of bias judgments

Domain 1: Study Participation	
a. Was there adequate participation in the study by eligible persons?	Y / N / U
b. Is the source population or population of interest adequately described?	Y / N
c. Are the baseline characteristics of the included mothers and babies adequately described?	Y / N
d. Is the period and site of recruitment adequately described?	Y / N
e. Are the inclusion and exclusion critieria adequately described?	Y / N
Rating	

◊ High Bias	The relationship between the index test and EONS is very likely to be different for participants and eligible non participants
◊ Moderate Bias	The relationship between the index test and EONS may be different for participants and eligible non participants
◊ Low Bias	The relationship between the index test and EONS is unlikely to be different for participants and eligible non participants

Domain 2: Study Attrition	
a. Were there any exclusions of mothers from the study after initially meeting inclusion criteria?	Y / N / U
b. Was there an attempt to collect information on participants who were excluded?	Y / N / U
c. Are the reasons for these exclusions / loss to follow up provided?	Y / N
d. Are the participants who were excluded / lost to follow up adequately described?	Y / N
e. Are there important differences between participants who completed the study and those who did not?	Y / N / U
Rating	

◊ High Bias	The relationship between the index test and EONS is very likely to be different for completing and non completing participants
◊ Moderate Bias	The relationship between the index test and EONS may be different for completing and non completing participants
◊ Low Bias	The relationship between the index test and EONS is unlikely to be different for completing and non completing participants

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Domain 3a: Prognostic Factor Measurement: Index Test (Complete a separate domain 3 for each index test assessed)	
a. Is a clear definition or description of the index test provided?	Y / N
b. Is the method of index test measurement adequately valid and reliable?	Y / N / U
c. Are predetermined cutoffs used?	Y / N / U
d. Is the method and setting of the index test the same for all study participants?	Y / N / U
e. Is there complete data for the index test for at least 90% of the study sample?	Y / N / U
f. If there is missing data, are appropriate methods of imputation used?	Y / N / n/a
Rating	

◊ High Bias	The measurement of the Index test is very likely to be different for different categories of the outcome: EONS
◊ Moderate Bias	The measurement of the Index test may be different for different categories of the outcome: EONS
◊ Low Bias	The measurement of the Index test is unlikely to be different for different categories of the outcome: EONS

Domain 3b: Prognostic Factor Measurement: Index Test (Complete a separate domain 3 for each index test assessed)	
a. Is a clear definition or description of the index test provided?	Y / N
b. Is the method of index test measurement adequately valid and reliable?	Y / N / U
c. Are predetermined cutoffs used?	Y / N / U
d. Is the method and setting of the index test the same for all study participants?	Y / N / U
e. Is there complete data for the index test for at least 90% of the study sample?	Y / N / U
f. If there is missing data, are appropriate methods of imputation used?	Y / N / n/a
Rating	

◊ High Bias	The measurement of the Index test is very likely to be different for different categories of the outcome: EONS
◊ Moderate Bias	The measurement of the Index test may be different for different categories of the outcome: EONS
◊ Low Bias	The measurement of the Index test is unlikely to be different for different categories of the outcome: EONS

Domain 4: Outcome Measurement	
a. Is there a clear definition and diagnostic criteria for the outcome?	Y / N
b. Is the method of outcome ascertainment used adequately valid and reliable?	Y / N / U
c. Is the method of outcome ascertainment the same for all study participants?	Y / N / U
d. Were the outcome assessors blinded to the results of the index test?	Y / N / U
Rating	
◊ High Bias	The ascertainment of the outcome, EONS is very likely to be different for different levels of the index test
◊ Moderate Bias	The ascertainment of the outcome, EONS may be different for different levels of the index test
◊ Low Bias	The ascertainment of the outcome, EONS is unlikely to be different for different levels of the index test

Domain 5: Study Confounding	
a. Are all important confounders measured?	Y / N / U
b. Are clear definitions of the important measured confounders given?	Y / N
c. Is the measurement of all important confounders adequately valid and reliable?	Y / N / U
d. Is the method and setting of confounding measurement the same for all study participants?	Y / N / U
e. Are appropriate imputation methods used for missing confounder data?	Y / N / n/a
f. Are important potential confounders accounted for in the study design?	Y / N / U
g. Are important potential confounders accounted for in the analysis?	Y / N / U
Rating	
◊ High Bias	The observed effect of the index test on EONS is very likely to be distorted by another factor related to the index test and EONS
◊ Moderate Bias	The observed effect of the index test on EONS may be distorted by another factor related to the index test and EONS
◊ Low Bias	The observed effect of the index test on EONS is unlikely to be distorted by another factor related to the index test and EONS

Domain 6: Statistical Analysis and Reporting	
a. Is there sufficient presentation of data to assess the adequacy of the analytic strategy?	Y / N
b. Is the selected statistical model adequate for the design of the study?	Y / N / U
c. Is there selective reporting of the results?	Y / N / U
◊ High Bias	The reported results are very likely to be spurious or biased related to analysis or reporting
◊ Moderate Bias	The reported results may be spurious or biased related to analysis or reporting
◊ Low Bias	The reported results are unlikely to be spurious or biased related to analysis or reporting

Appendix 4b. QUIPS Rating Guidance Tool

Inflammatory Markers for PPROM, Systematic Review, QUIPS Rating Guidance tool

Name of Reviewer Completing the Form _____

Date form completed_____

Study ID (Author/Year) _____/_____ **Citation ID(s)** _____

Key

Y – Yes, N – No, U- Unclear, n/a – Not applicable

QUIPS tool: Risk of bias judgments

Domain 1: Study Participation	
a. Was there adequate participation in the study by eligible persons?	Y / N / U
b. Is the source population or population of interest adequately described?	Y / N
c. Are the baseline characteristics of the included mothers and babies adequately described?	Y / N
d. Is the period and site of recruitment adequately described?	Y / N
e. Are the inclusion and exclusion criteria adequately described?	Y / N
Optimal characteristic: The study sample adequately represents the population of interest. **High Risk of Bias – low participation rate; sample size has a very different age and sex distribution from the source population; or a very selective rather than consecutive sample of eligible patients was recruited.** **Low Risk of Bias - high participation of eligible and consecutively recruited patients; recruited patients have characteristics similar to those in the source population.**	
Rating	

◊ High Bias	The relationship between the index test and EONS is very likely to be different for participants and eligible non participants
◊ Moderate Bias	The relationship between the index test and EONS may be different for participants and eligible non participants
◊ Low Bias	The relationship between the index test and EONS is unlikely to be different for participants and eligible non participants

Domain 2: Study Attrition	
a. Were there any exclusions of mothers from the study after initially meeting inclusion criteria?	Y / N / U
b. Was there an attempt to collect information on participants who were excluded?	Y / N / U
c. Are the reasons for these exclusions / loss to follow up provided?	Y / N
d. Are the participants who were excluded / lost to follow up adequately described?	Y / N
e. Are there important differences between participants who completed the study and those who did not?	Y / N / U
Optimal characteristic: The study data available (ie participants not lost to follow up) adequately represents the study sample. **High Risk of Bias – Persons who completed the study are likely to differ from those lost to follow-up in a way that distorts the association between the prognostic factor and outcome.** **Low risk of Bias – Study has complete follow up or evidence of participants missing at random.**	

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Rating		
◊ High Bias	The relationship between the index test and EONS is very likely to be different for completing and non completing participants	
◊ Moderate Bias	The relationship between the index test and EONS may be different for completing and non completing participants	
◊ Low Bias	The relationship between the index test and EONS is unlikely to be different for completing and non completing participants	
Domain 3a: Prognostic Factor Measurement: Index Test (Complete a separate domain 3 for each index test assessed)		
a. Is a clear definition or description of the index test provided?		Y / N
b. Is the method of index test measurement adequately valid and reliable?		Y / N / U
c. Are predetermined cutoffs used?		Y / N / U
d. Is the method and setting of the index test the same for all study participants?		Y / N / U
e. Is there complete data for the index test for at least 90% of the study sample?		Y / N / U
f. If there is missing data, are appropriate methods of imputation used?		Y / N / n/a
Optimal characteristic: The prognostic factor is measured in a similar way for all participants. **High risk of bias – an unreliable method was used to measure the index test; or different approaches were used to measure the index test in different participants resulting in systematic misclassification.** **Low risk of bias – the index test is measured similarly for all participants and uses a valid, reliable measure.**		
Rating		
◊ High Bias	The measurement of the Index test is very likely to be different for different categories of the outcome: EONS	
◊ Moderate Bias	The measurement of the Index test may be different for different categories of the outcome: EONS	
◊ Low Bias	The measurement of the Index test is unlikely to be different for different categories of the outcome: EONS	
Domain 4: Outcome Measurement		
a. Is there a clear definition and diagnostic criteria for the outcome?		Y / N
b. Is the method of outcome ascertainment used adequately valid and reliable?		Y / N / U
c. Is the method of outcome ascertainment the same for all study participants?		Y / N / U
d. Were the outcome assessors blinded to the results of the index test?		Y / N / U
Optimal characteristic – The outcome of interest is measured in a similar way for all participants. **Low risk of bias – If the outcome is measured similarly for all participants and uses a valid and reliable measure.** **High risk of bias – There is likely to be a differential measurement of outcome related to the or result of the index test. Eg. If babies whose mothers had positive results on the index test were more thoroughly evaluated for neonatal sepsis.**		
Rating		
◊ High Bias	The ascertainment of the outcome, EONS is very likely to be different for different levels of the index test	

◊ Moderate Bias	The ascertainment of the outcome, EONS may be different for different levels of the index test
◊ Low Bias	The ascertainment of the outcome, EONS is unlikely to be different for different levels of the index test

Domain 5: Study Confounding	
a. Are all important confounders measured?	Y / N / U
b. Are clear definitions of the important measured confounders given?	Y / N
c. Is the measurement of all important confounders adequately valid and reliable?	Y / N / U
d. Is the method and setting of confounding measurement the same for all study participants?	Y / N / U
e. Are appropriate imputation methods used for missing confounder data?	Y / N / n/a
f. Are important potential confounders accounted for in the study design?	Y / N / U
g. Are important potential confounders accounted for in the analysis?	Y / N / U
Optimal characteristic: Important potential confounding factors are appropriately accounted for. **High risk of bias – another factor related to both the prognostic factor and the outcome is likely to explain the effect of the prognostic factor.** **Low risk of bias – Adequate measurement of important potential confounding variables and inclusion of these variables in a pre-specified multivariable analysis.** **We shall consider important confounders to be antibiotic use and gestation age.**	
Rating	

◊ High Bias	The observed effect of the index test on EONS is very likely to be distorted by another factor related to the index test and EONS
◊ Moderate Bias	The observed effect of the index test on EONS may be distorted by another factor related to the index test and EONS
◊ Low Bias	The observed effect of the index test on EONS is unlikely to be distorted by another factor related to the index test and EONS

Domain 6: Statistical Analysis and Reporting	
a. Is there sufficient presentation of data to assess the adequacy of the analytic strategy?	Y / N
b. Is the selected statistical model adequate for the design of the study?	Y / N / U
c. Is there selective reporting of the results?	Y / N / U
Optimal characteristic: The statistical analysis is appropriate and all primary outcomes are reported. **Low risk of bias – statistical analysis appropriate for the data, statistical assumptions are satisfied and all primary outcomes are reported.**	

◊ High Bias	The reported results are very likely to be spurious or biased related to analysis or reporting
◊ Moderate Bias	The reported results may be spurious or biased related to analysis or reporting
◊ Low Bias	The reported results are unlikely to be spurious or biased related to analysis or reporting

Appendix 5. Aga Khan University (Nairobi), Health Research Committee Exemption from Ethical Review

THE AGA KHAN UNIVERSITY

Faculty of Health Sciences
Medical College

Ref: 2015/REC-33(v2)
11th August 2015

Dr. Angela Koech
Resident- Department of Obstetrics and Gynaecology,
Aga Khan University-EA, Nairobi

Dear Dr. Koech,

Re: MATERNAL INFLAMMATORY MARKERS IN THE DIAGNOSIS OF CHORIOAMNIONITIS AND PREDICTION OF NEONATAL SEPSIS IN PRETERM PRE-LABOUR RUPTURE OF MEMBRANES: A SYSTEMATIC REVIEW

The Aga Khan University, Nairobi Health Research Ethics Committee (REC) is in receipt of your proposal and application submitted to the Research Support Unit (RSU) on 04th August 2015. In a meeting held on 10th August 2015, the committee reviewed your application and recorded that the proposed study is a systematic review of published studies.

The committee thus approved your request for exemption from a protracted ethics review process. This proposal is also in compliance with the Aga Khan University Research Ethics Regulations. You are authorized to conduct this study from **14th August 2015**. This approval is valid until **13th August 2016.**

The study should be conducted in full accordance with all the applicable sections of the R&EC guidelines. You must request extension if additional time is required for study completion. As the principal investigator you must advise the R&EC when this study is finished or discontinued and a final report submitted to the RSU. If you have any questions, please contact Research Support Unit - kamanda.ciru@aku.edu or 020-366 2148.

Sincerely,

Dr. Amyn Lakhani, Chair
Health Research Ethics Committee, AKU (N)

Appendix 5. Characteristics of Excluded Studies

Study	Country	Study Design	Gestational Age Range (weeks)	Index Test(s), and cut-off	Reference Standard / Outcome	Reason for Exclusion
Evans 1980	USA	Prospective cohort	≤36	CRP 2mg/dL	HCA, Infectious morbidity	No 2x2 data for CRP vs HCA in PPROM subgroup (Study population includes term PROM, term and preterm labour, composite outcome of infectious morbidity)
Ernest 1987	USA	Prospective cohort	26 - 34	CRP, 0.8mg/dL, 2mg/dL	Neonatal Sepsis, 3 days	No 2x2 data for CRP vs EONS in PPROM subgroup (Study population includes preterm labour)
Watts 1993	USA	Prospective cohort	22 -34	CRP, 1.5mg/dL	HCA	No 2x2 data for PPROM subgroup (Study population includes preterm labour)
Murtha 1996	USA	Prospective cross-sectional	22 -34	IL6, 8pg/mL	HCA	No 2x2 data for IL6 vs HCA
Pfeiffer 1999	Germany	prospective	Any	IL6, 11pg/mL CRP 1.2mg/dL	Perinatally acquired neonatal infection, 48 hours	No 2x2 data for subgroup with PPROM (Study population includes term PROM)
Zou 2004	China	Prospective cohort	20 - 37	CRP, 1.03mg/dL	HCA	No 2x2 data for PPROM subgroup (Study population includes term PROM)
Skrablin 2007	Croatia	Prospective cohort	27-33	CRP, 10.8mg/L IL6, 27.5pg/mL	HCA Connatal infection, includes early onset clinical sepsis	No 2x2 data for PPROM subgroup (Study population includes preterm labour)
Yinon 2007	Israel	Prospective cohort	24 - 35	CRP, cut-off not provided	HCA (and funisitis)	No 2x2 data for CRP vs HCA
Debieve 2010	Belgium	Prospective Cohort	24 -35	CRP, 1mg/dL	HCA Neonatal Sepsis	No 2x2 data for CRP vs HCA Neonatal sepsis – Not early
Oh 2011	Korea	Retrospective cohort	21 -35 (at birth)	CRP, 0.6mg/dL	HCA	No 2x2 data for subgroup with PPROM
Popowski 2011	France	Prospective Cohort	>34 weeks	CRP, 5mg/L	HCA, Early Onset Neonatal Infection, 72 hours	No 2x2 data for PPROM subgroup (Study population includes term PROM)
Lee 2012	Korea	Retrospective Cohort	<36	CRP 8mg/L (4,8,12,20))	HCA EONS	No 2x2 data for the subgroup with PPROM
Mercer 2012	USA	Prospective cohort (from a trial)	24 -32	IL6, No cut-off provided	EONS, 72 hours	No 2x2 data for IL6 vs EONS
Wang 2012	China	Prospective cohort		CRP, 4.4mg/L	HCA	No 2x2 data for PPROM subgroup (Study population includes term PROM)

Appendix 5 continued, Characteristics of Excluded Studies

Study	Country	Study Design	Gestational Age Range (weeks)	Index Test(s), and cut-off	Reference Standard / Outcome	Reason for Exclusion
Cekmez 2013a	Turkey	Prospective Cohort	24-34	CRP, 10.2pg/mL IL6, 9.5pg/mL	HCA, ? Infectious morbidity	No 2x2 data for PPROM subgroup (Unclear whether available data includes normal pregnant controls and whether it refers to HCA only or a composite outcome)
Cekmez 2013b	Turkey	Prospective cohort	24-34	CRP 10.3pg/mL IL6 9.6pg/mL	HCA, ?Infectious morbidity	Unclear whether 2x2 data refers to HCA only or a composite outcome
Gveric-Ahmetasevic 2014	Croatia	Prospective cohort		CRP, 7mg/L, PCT 0.053ng/L	Early neonatal onset bacterial infection, 48 hours	No 2x2 data for PPROM subgroup (Study population includes term PROM)
Jeon 2014	Korea	Retrospective Cohort	Preterm, <37 weeks	CRP, 1.22mg/dL	HCA EONS, 3 days	No 2x2 data for the subgroup with PPROM
Kim 2014	Korea	Retrospective Cohort	24 -37	CRP, 7.46mg/L	HCA and funisitis	No 2x2 data for the subgroup with PPROM
Park 2014	Korea	Prospective Cohort	Preterm (at birth)	CRP, 0.7ng/mL	HCA EONS, 72 hours	No 2x2 data for subgroup with PPROM
Xie 2015	China	Retrospective cohort	<34	CRP, 8mg/L	HCA EONS, 72 hours	No 2x2 data CRP vs HCA or EONS
Kwak 2015	Korea	Prospective Cohort	≤ 37	CRP, 0.8mg/dL	HCA	Unclear / Conflicting 2x2 data