DIAGNOSIS OF INTRAUTERINE INFLAMMATION/INFECTION IN WOMEN WITH PRETERM PRELABOR RUPTURE OF MEMBRANES USING CERVICOVAGINAL INTERLEUKIN-6 CONCENTRATIONS: SYSTEMATIC REVIEW AND META-ANALYSIS

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Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

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ABSTRACT

Title: Diagnosis of intrauterine inflammation/infection in women with preterm prelabor rupture of membranes using cervicovaginal interleukin-6 concentrations: systematic review and meta-analysis

Objective: To establish the diagnostic test accuracy of cervicovaginal interleukin-6 concentration in the detection of intraamniotic inflammation, in women with preterm prelabor rupture of membranes (PPROM) at less than 37 weeks of gestational age.

Data sources: A systematic literature search was undertaken using Embase, SCOPUS, PubMed, and the Cochrane library from their inception to Feb 2022.

Study eligibility criteria: Prospective and retrospective studies evaluating cervicovaginal interleukin-6 (IL-6) concentrations, in women diagnosed with PPROM at less than 37weeks of gestation, were included.

Study appraisal and synthesis methods: Cervicovaginal IL-6 concentration was assessed as the index test for the prediction of intraamniotic inflammation. The Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) was independently used by two reviewers to assess the quality of the studies. Forest plots for sensitivity and specificity with 95% CIs were constructed. Hierarchical summary receiver operating characteristic curves were constructed; quantitative data synthesis was performed using random-effects models. Diagnostic odds ratios were calculated to measure the global effectiveness of the diagnostic test. Heterogeneity was

assessed using Cochran's Q statistic, with $p < 0.10$ denoting heterogeneity. Meta-regression was performed to assess the effect of 2 covariates. Publication bias could not be assessed given the limited number of studies.

Results: Eighteen studies were retained for qualitative analysis, 14 were included in the meta-analysis. The study population included 1,655 women diagnosed with PPROM. According to the QUADAS-2 tool, all included studies were of poor quality. The area under the curve (AUC) for the diagnosis of intraamniotic inflammation and/or infection by cervicovaginal IL-6 was 0.838 ± 0.035. The pooled diagnostic odds ratio of cervicovaginal IL-6 was found to be 10.74 (95% CI 5.45, 21.17). Based on the Cochran's Q value of 88.03 ($p = 0.00$), a high degree of heterogeneity exists across studies.

Conclusions: Cervicovaginal IL-6 has a DOR of 10.74 (95% CI 5.45, 21.17) in the detection of intrauterine inflammation/infection. Based on our results, cervicovaginal IL-6 seems to have a good diagnostic accuracy in the detection of intraamniotic inflammation/infection in women with PPROM.

Keywords: cervicovaginal, chorioamnionitis, inflammatory markers, interleukin-6, intraamniotic inflammation, preterm prelabor rupture of membranes

INTRODUCTION

1.1 Background: In the United States, where preterm birth (PTB) is the leading cause of neonatal mortality, approximately 1 in 3 cases are precipitated by preterm prelabor rupture of membranes (PPROM).[1] PPROM describes rupture of the amniotic sac prior to 37 weeks of gestation and before the onset of regular uterine contractions.[2] It is a serious pregnancy complication that significantly increases the risk of infection and premature birth.[2]

1.2 PPROM Etiology: The pathophysiology of PPROM has yet to be established, though there are various hypotheses regarding causative etiologies.[2–4] One of the proposed mechanisms is an increase in proinflammatory cytokines resulting in intraamniotic inflammation (IAI).[2,5–7] IAI is either sterile or associated with microbial invasion of the amniotic cavity (MIAC).[8–10] The development of microbial associated IAI is due to a tightly orchestrated inflammatory response.[11–13] The inflammatory response is initiated by the activation of pattern recognition receptors (PRRs) by a specific pattern on the surface of microbial cells.[11–13] The same system of receptors may be activated by endogenous molecules called alarmins, which are released from membranes of necrotic fetal cells and lead to sterile IAI.[11–13] Inflammation is thought to cause collagenolysis via disruption of the matrix metalloproteinase (MMP)/ tissue-specific inhibitors of metalloproteinases (TIMPs) balance, though the exact mechanism has yet to be elucidated.[14]

1.3 Clinical Sequelae

Inflammation in the setting of PPROM substantially increases the risk of preterm delivery.[7] Preterm delivery, and its sequelae are negatively correlated with latency (time from PPROM to delivery) with shorter latencies resulting in increased neonatal morbidity and mortality.[2]

Regardless of the etiology IAI is associated with adverse pregnancy and neonatal outcomes.[15–17] It can trigger a fetal inflammatory response and may lead to development of fetal inflammatory response syndrome.[18,19] FIRS is defined as an increase in the inflammatory reaction in the fetus with or without infection.[18] It influences the chances of the newborn's survival and in the long term, the child's developmental potential.[20] FIRS has been demonstrated to damage the fetal brain, kidney, and negatively impact heart function.[20]

1.4 Diagnosis of intraamniotic inflammation/infection

A high index of suspicion is needed to diagnose intraamniotic infection promptly since early signs and symptoms can be subtle.[2] Gibbs criteria[21] have traditionally been used to diagnose clinical chorioamnionitis but these criteria have low sensitivity and specificity.[10,22,23] Signs of inflammation and infection can be detected through analysis of amniotic fluid obtained via an amniocentesis. However, amniocentesis is an invasive test and may not be feasible due to oligohydramnios (low level of amniotic fluid), lack of provider experience, or patient refusal. The success rate for an amniocentesis in the setting of PPROM is reported to be between 45 & 97% depending on the availability of an accessible fluid pocket.[24] Subsequently, a non-invasive test able to evaluate for intraamniotic inflammation would obviate the need for an invasive procedure and be clinically useful in determining the presence of inflammation. Intrauterine inflammation associated with PPROM is therefore a target of intense investigational interest.

1.5 Interleukin-6

Several studies have identified interleukin-6 (IL-6) as a sensitive marker of intraamniotic inflammation.[25–27,27–29] The pleotropic cytokine, has been shown to be an efficient marker of IAI

and MIAC that is not inferior to modern proteomic markers.[25–28] Furthermore, IL-6 concentrations analyzed from vaginal fluid have been shown to have a high negative predictive value for the detection of microbial-associated intraamniotic inflammation, intraamniotic inflammation, and microbial invasion of the amniotic cavity.[1]

1.6 Rationale for a systematic review and meta-analysis of diagnostic test accuracy

Though several studies have been conducted to investigate the diagnostic power of cervicovaginal IL-6 in the setting of PPROM, there is no study to date, aggregating the findings. Currently, IL-6 remains the most studied biomarker for chorioamnionitis though no consensus has been reached regarding its sensitivity, specificity, or accuracy for the presence of intrauterine inflammation and/or infection.[30–32]

2. MANUSCRIPT

2.1 Introduction

Preterm prelabor rupture of membranes (PPROM) describes rupture of the amniotic sac prior to 37 weeks of gestation and before the onset of regular uterine contractions.[2] It complicates 2-3% of all pregnancies and is responsible for one in three preterm births (PTB) in the United States.[1] PTB is the leading cause of neonatal mortality in the US and a risk factor for neonatal disability with an annual cost of approximately $26.2 billion.[3,4] In addition to its association with PTB, PPROM is implicated in 15-35% of cases of intraamniotic infection.[2] Intraamniotic infection, even without the presence of maternal symptoms, can lead to fetal inflammatory response syndrome (FIRS), a condition resulting in higher rates of cerebral palsy, intraventricular hemorrhage, sepsis, respiratory distress syndrome, necrotizing enterocolitis, and neurodevelopmental disorders.[18,33–36] Interestingly, sterile intrauterine inflammation (without microbial involvement) has also been shown to lead to poor perinatal outcomes.[1,9,15–17,37,38] Given the multitude of morbid sequelae, PPROM is a serious pregnancy complication that deserves research attention.

The management of PPROM involves balancing the risk of prematurity with the risk of infection.[2,39] Expectant management is usually favored prior to 34 weeks of gestation unless there are overt signs of fetal distress, labor, or infection.[2] However, detecting early-stage infections in utero is difficult.[10,22,23,33] Consequently, in cases when expectant management is pursued, infections often progress until women develop clinical features, at which time neonatal outcomes are adversely impacted.[2,40]

Currently we rely on the use of Gibbs clinical criteria to monitor women with PPROM.[2,21,41] When there is increased suspicion of infection, an amniocentesis is performed.[41] However, Gibbs criteria has low specificity for intrauterine infection and an amniocentesis is invasive, precluding longitudinal evaluation.[2,21,41] Additionally, amniocentesis requires an expert operator, may not be possible due to lack of an accessible fluid pocket, and the culture results (most likely polymicrobial) are not available for several days.[42] Therefore, levels of other less sensitive and specific markers, in the amniotic fluid, including gram staining, glucose levels, white blood cell count, and leukocyte esterase, are used clinically to deduce if intraamniotic infection is present.[27,41,42]

Increasingly, inflammatory markers have shown promise in the early detection of chorioamnionitis. In particular, amniotic fluid interleukin-6 (IL-6) concentration has emerged as an important cytokine for the identification of intraamniotic inflammation/infection.[2,5,6,25–28,43] Some of the promising results of amniotic fluid IL-6 have been translated into noninvasive sampling using cervicovaginal fluid.[24,44–46] IL-6 concentrations in vaginal fluid have been posited to have a high negative predictive value for the detection of microbial-associated intraamniotic inflammation and microbial invasion of the amniotic cavity (MAIC).[24,44–46]

Currently, IL-6 remains the most studied biomarker for chorioamnionitis though no consensus has been reached regarding its sensitivity, specificity, or accuracy for the presence of intrauterine inflammation and/or infection.[14,30,32] The objective of this systematic review is to aggregate the findings of all the studies on this subject in order to determine the diagnostic accuracy of cervicovaginal IL-6 in the detection of intraamniotic inflammation, microbial invasion of the

amniotic cavity, histologic chorioamnionitis, fetal inflammatory syndrome, funisitis, and early onset neonatal sepsis in women with preterm prelabor rupture of membranes at less than 37 weeks of gestational age.

2.2 Methods

2.2.a Eligibility criteria, information sources, and search strategy

A comprehensive literature search was conducted, with the aid of a librarian, using Embase, SCOPUS, PubMed, and the Cochrane library from database inception through Feb of 2022. No language restrictions were applied to the electronic search.

The review was conducted in accordance with the methodological approaches listed in the Cochrane Diagnostic Test Accuracy Review guidelines.[47] The protocol was agreed on by all authors and registered with the international Prospective Register of Systematic Reviews (CRD42022332119). The final report was written according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy Studies (PRISMA-DTA) statement.[48]

The initial screening of titles and abstracts was conducted by one investigator (D.G) and verified by a second (C.C.). In-depth review of full texts, meeting inclusion criteria, was independently undertaken by both investigators, (D.G.) and (C.C). Disputes were resolved through discussion or consultation with a third investigator (K.G.). Despite not including language restrictions in the electronic search and abstract screening, anticipated translation difficulties lead to the

exclusion of non-English full texts. A detailed description of the search strategy and query syntax is listed in Supplemental Table 1.

2.2.b Study selection

We included observational studies (prospective or retrospective), in women diagnosed with PPROM at less than 37 weeks of gestation, that evaluated cervicovaginal IL-6 concentrations in relation to intrauterine infection and/or inflammation.

2.2.c Data Extraction

Two reviewers (D.G. and C.C.) independently extracted data from the included studies using a predetermined data extraction form. Prior to its use, the form was piloted on three randomly selected studies to ensure usability. The following data were extracted on the data sheet: first author, publication year, study design, participant characteristics (mean maternal age at inclusion, mean gestational age at diagnosis and delivery), index test characteristics (method of collection and sample analysis), IL-6 concentration thresholds, sampling method including (frozen vs. fresh), clinical management (use of antibiotics, steroids, tocolytics, and magnesium), and information on the reference tests used.

2.2.d Assessment of risk of bias

The Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) was utilized to assess the quality of the studies in four domains, patient selection, index test, reference standard, and flow and timing.[49] Each study was independently assessed by two investigators (D.G and C.C.) Disputes were settled through discussion and consultation with a third investigator (K.G.).

Answers were codified as low risk, unclear, or high risk based on the judgement of investigators and the results were assessed and graphed using the Review Manager (RevMan) computer program (version 5.4 The Cochrane Collaboration 2020).

2.2.e Statistical Analysis

A 2x2 table was created with data extracted or calculated from articles that compared the results of the index test to any of the predefined reference standards. The table was then used to calculate the sensitivity, specificity, and 95% confidence intervals of the index test at the various thresholds specified in the studies. Results were presented as coupled forest plots. Meta-analysis was carried out if the number of studies in each reference test category was ≥3. Extracted results were pooled in a meta-analysis, and hierarchical summary receiver-operating characteristic (hSROC) curves were constructed. Models were fitted using the Rutter and Gastonis model and the random effects model was used for quantitative data synthesis.

Outcomes (reference standards) evaluated were IAI (defined as the presence of [IL-6] > 745pg/mL in the amniotic fluid)[1,50,51], MIAC (defined as positive amniotic fluid culture or PCR)[1,24,44–46,50–52], HCA (defined as the presence of neutrophil infiltration in the chorion or amnion)[33,39,46,52–56], FIRS (defined as elevated IL-6 concentration in cord plasma at the time of birth)[36,39], funisitis (defined as the presence of neutrophil infiltration into the wall of the umbilical vein and or/arteries and/or Wharton's jelly)[57,58], and EONS (defined as a positive blood or cerebrospinal fluid culture in the first 72hrs of life or suspected clinical sepsis diagnosed by a neonatologist based on clinical symptoms)[39,42,54,56,59,60]. Each reference standard was assessed by

the index test, cervicovaginal IL-6 concentration. Meta-regression (weighted linear regression) analysis was performed to assess the effect of gestational age (<34 weeks vs. ≥ 34 weeks).

Diagnostic odds ratios (DORs, positive likelihood ratio/negative likelihood ratio) were calculated to measure the global effectiveness of the diagnostic test, cervicovaginal IL-6. The heterogeneity of combined values of sensitivity and specificity was assessed using Cochran's Q statistic, with $p < 0.10$ denoting heterogeneity.

Publication bias could not be assessed given the limited number of studies and the large amount of heterogeneity. Between-study heterogeneity was visually assessed (the further the points were from the curve, the larger the heterogeneity present). A sensitivity analysis was not done. Meta-DiSc (version 2.0) was used to conduct all statistical analyses.

2.3 Results

2.3.a Study selection

After excluding 129 duplicates, our search identified 796 potentially relevant studies. Of the 796 identified, 33 studies were eligible for full-text-review. Our manual search did not identify additional studies meeting criteria. Upon full-text review, 18 studies were retained for qualitative analysis.[1,24,33,36,39,42,44–46,50–56,59,60] Fourteen of the 18, were included in the meta-analysis.[1,24,33,39,42,44,45,50,53–56,59,60] The review flow diagram is depicted in Figure 1. The characteristics of the included and excluded studies are listed in Tables 1 & 2, respectively.

2.3.b Risk of bias of the included studies

Figures 2a. and 2b. tabulate the risk of bias of the included studies according to the QUADAS-2 tool for diagnostic test accuracy reviews.[49] In the 'patient selection' domain, we judged 2 of the 18 studies to be at high risk of bias due to inappropriate patient exclusions and case-control study design.[33,39] One study, demonstrated an unclear risk of bias in the 'patient selection domain' due to inappropriate patient exclusions.[24] When assessing the 'index test' domain, studies where the cut-offs used were not prespecified, rather determined from the study data, were deemed to be at high risk of bias.[1,24,33,36,39,44–46,52,55,59,60] There was no concern for bias in the 'reference standard' domain. Reference standards were interpreted without knowledge of the index test results in all studies. In the 'flow and timing' domain, we assumed a ≤72h interval, between cervicovaginal sampling and delivery, to be appropriate in the preservation of the relationship between the index and reference test results. This did not apply to studies using MIAC as the reference standard since amniotic fluid and cervicovaginal fluid were collected and analyzed at the same time. Subsequently, 13 studies[1,24,33,36,39,42,50,52,54–56,59,60] were judged to be of unclear risk, and one study was considered high risk in the 'flow and timing domain'. The high-risk study did not include all patients in the analysis in addition to having an uncertain interval between the index and reference tests.[46] Regarding applicability, all but one study[36] included were considered low risk. One study had an unclear bias in the 'patient selection' domain; the population did not match the study criteria exactly. Overall, the included studies were of poor quality with all but six[42,50,51,53,54,56] determined to be at high risk of bias in at least one domain.

2.3.c <u>Synthesis of results</u>

The 14 studies included in the meta-analysis were published between 1998 and 2021.[1,24,33,39,42,44,45,50,53–56,59,60] The studies included 1,303 women diagnosed with PPROM. Six of the studies included women with PPROM at less than 34 weeks of gestational age.[33,39,42,45,55,59] The remaining 8 studies additionally included women with PPROM between 34 and 37 weeks of gestational age.[1,24,44,50,53,54,56,60] Five studies included cervicovaginal IL-6 as the index test and MIAC as the reference test.[1,24,44,45,50] These 5 studies included 545 women, 150 of them with MIAC.[33,53–56] The median prevalence of MIAC in the 5 studies was 26% with an interquartile range of 22% to 37%. Six studies contained information regarding cervicovaginal IL-6 concentrations and HCA. The 6 studies included 544 women, 268 of which had HCA. The median prevalence of HCA in the 6 studies was 47% with an interquartile range of 40% to 63%. Finally, 6 studies with 512 women, 133 with infants diagnosed with EONS, assessed cervicovaginal IL-6 concentrations and EONS as a reference test.[39,42,54,56,59,60]The median prevalence of EONS in these studies was 22% with an interquartile range of 18% to 37%.

Results on the global diagnostic performance of cervicovaginal IL-6 concentration for the prediction of intraamniotic inflammation/infection as well as stratified results with MIAC, HCA, and EONS as reference standards are presented in the main text below while the sensitivities, specificities, and 95% CI of IL-6 in the diagnosis of IAI, FIRS, MIAC+IAI, & MIAC+HCA are listed in supplemental figures 1a, 1b, 1c, and 1d. The latter were not included in the meta-analysis due to insufficient data (< 3 studies in each category).

2.3.d <u>Global diagnostic test assessment</u>

The studies analyzed reported a wide range of index test cutoffs (100-50,000 pg/mL). Due to the multiple thresholds, a summary point was deemed an inappropriate representation of the diagnostic test accuracy of cervicovaginal IL-6.[47] Figure 4 depicts an hSROC curve with each study plotted as a single sensitivity-specificity point on the curve; the scatter is demonstrative of the heterogeneity of the findings. The area under the curve (AUC) for the diagnosis of intraamniotic inflammation and/or infection by cervicovaginal IL-6 was 0.838 ± 0.035.

Table 3 and Figure 5 depict the DOR of cervicovaginal IL-6 for the detection of intraamniotic inflammation/infection from each study. The pooled DOR of cervicovaginal IL-6 was found to be 10.74 (95% CI 5.45, 21.17). Based on the Cochran's Q value of 88.03 (p = 0.00), a high degree of heterogeneity exists between the sensitivity and specificity of cervicovaginal IL-6 in the detection of intrauterine inflammation/infection across studies.

To explore a biologically plausible cause of between study variation, a meta-regression to apply gestational age (<34wks v <37wks) as a covariate to the global assessment of cervicovaginal IL-6 as a diagnostic tool for the detection of intraamniotic inflammation/infection was undertaken (figures 6a & 6b). Eight studies included patients with a GA <34 weeks ranging in sensitivity from 0.33 (95% CI 0.15, 0.57) to 1.00 (95% CI 0.86, 1.00) and specificity ranging from 0.48 (95% CI 0.34-0.63) to 0.95 (95% CI 0.87, 0.99)[33,39,42,45,53,55,56,59] while 6 studies included patients with GA up to 37 weeks with sensitivity ranging from 0.36 (95% CI 0.18, 0.57) to 1.00 (95% CI 0.66, 1.00), and specificity ranging from 0.29 (95% CI 0.21, 0.39) to 0.89 (95% CI 0.82, 0.95).[1,24,44,50,54,60]

2.3.e MIAC as reference standard

A paired forest plot depicting the sensitivities, specificities, and corresponding 95% CIs of cervicovaginal IL-6 in the diagnosis of MIAC is presented in Figure 3a. The forest plot (figure 3a) shows a sensitivity ranging from 0.36 (95% CI 0.18, 0.57) to 0.92 (95% CI 0.62, 1.00) and a specificity ranging from 0.71 (95% CI 0.54, 0.84) to 0.89 (95% CI 0.82, 0.95) respectively.

A total of 5 studies included information on the use of cervicovaginal IL-6 in the diagnosis of MIAC.[1,24,44,45,50] The studies analyzed reported a wide range of index test cutoffs (200-2,500 pg/mL). Due to the multiple thresholds, a summary point was deemed an inappropriate representation of the diagnostic test accuracy of cervicovaginal IL-6.[47] SROC curves were constructed with each study plotted as a single sensitivity-specificity point on the curve; the scatter is demonstrative of the heterogeneity of the findings.

To explore clinically relevant causes of between study variation, the effect of GA as a covariate was explored. Figures 7a and 8a depict the results of a meta-regression with GA (< 34 or <37 wks) fit as a covariate in the detection of MIAC by cervicovaginal IL-6. One study included patients with a GA <34 weeks; sensitivity 0.79 (95% CI 0.63, 0.90) and specificity 0.73 (95% CI 0.62, 0.82).[45] The 4 studies that included patients with GA up to 37 weeks had a sensitivity of 0.36 (95% CI 0.18, 0.57) to 0.92 (95% CI 0.62, 1.00), and a specificity of 0.71 (95% CI 0.54, 0.84) to 0.89 (95% CI 0.82, 0.95).[1,24,44,50]

2.3.f HCA as reference standard

A paired forest plot of sensitivities, specificities, and the corresponding 95% CIs of cervicovaginal IL-6 in the diagnosis of HCA is presented in Figure 3b. The forest plot (figure 3b) shows a sensitivity ranging from 0.60 (95% CI 0.49, 0.71) to 1.00 (95% CI 0.86, 1.00) and specificity ranging from 0.67 (95% CI 0.53, 0.79) to 0.91 (95% CI 0.72, 0.99) respectively in the detection of HCA by cervicovaginal IL-6 in women with PPROM.

A total of 6 studies included information on the use of cervicovaginal IL-6 in the diagnosis of HCA.[33,39,53–56] Theses studies reported a wide range of index test cutoffs (100-50,000 pg/mL). Due to the multiple thresholds, a summary point was deemed an inappropriate representation of the diagnostic test accuracy of cervicovaginal IL-6.[47] SROC curves were constructed with each study plotted as a single sensitivity-specificity point on the curve; the scatter is demonstrative of the heterogeneity of the findings.

To explore clinically relevant causes of between study variation, the effect of GA as a covariate was explored. Figures 7b and 8b depict the results of a meta-regression with GA (< 34 or <37 wks) fit as a covariate in the detection of HCA by cervicovaginal IL-6. Three studies included patients with a GA <34 weeks ranging in sensitivity from 0.65 (95% CI 0.41, 0.85) to 0.88 (95% CI 0.77, 0.95) and specificity ranging from 0.69 (95% CI 0.39-0.91) to 0.76 (95% CI 0.50, 0.93).[33,39,55] The 3 studies that included patients with GA up to 37 weeks had a sensitivity of 0.60 (95% CI 0.49, 0.71) to 1.00 (95% CI 0.86, 1.00), and a specificity of 0.67 (95% CI 0.53, 0.79) to 0.91 (95% CI 0.72, 0.99).[53,54,56]

2.3.g <u>EONS as reference standard</u>

A paired forest plot of sensitivities, specificities, and the corresponding 95% CIs of cervicovaginal IL-6 in the diagnosis of EONS is presented in Figure 3c. The forest plot (figure 3c) shows a sensitivity ranging from 0.33 (95% CI 0.15, 0.57) to 1.00 (95% CI 0.66, 1.00) and specificity ranging from 0.35 (95% CI 0.14, 0.62) to 0.93 (95% CI 0.80, 0.98), respectively in the detection of EONS by cervicovaginal IL-6 in women with PPROM.

A total of 6 studies included information on the use of cervicovaginal IL-6 in the diagnosis of EONS.[39,42,54,56,59,60] Theses studies reported a wide range of index test cutoffs (100-50,000 pg/mL). Due to the multiple thresholds, a summary point was deemed an inappropriate representation of the diagnostic test accuracy of cervicovaginal IL-6.[47] SROC curves were constructed with each study plotted as a single sensitivity-specificity point on the curve; the scatter is demonstrative of the heterogeneity of the findings.

To explore clinically relevant causes of between study variation, the effect of GA as a covariate was explored. Figures 7c and 8c depict the results of a meta-regression with GA (< 34 or <37 wks) fit as a covariate in the detection of EONS by cervicovaginal IL-6. Three studies included patients with a GA <34 weeks ranging in sensitivity from 0.33 (95% CI 0.15, 0.57) to 0.95 (95% CI 0.75, 1.00) and specificity ranging from 0.48 (95% CI 0.34-0.63) to 0.95 (95% CI 0.87, 0.99).[39,42,59] The 3 studies that included patients with GA up to 37 weeks had a sensitivity of 0.59 (95% CI 0.39, 0.76) to 1.00 (95% CI 0.66, 1.00), and a specificity of 0.29 (95% CI 0.21, 0.39) to 0.89 (95% CI 0.71, 0.98).[54,56,60]

2.4 Comment

Intrauterine inflammation whether sterile or microbial-associated leads to adverse pregnancy and neonatal outcomes.[8–10,15–17] The development of microbial-associated IAI is due to a tightly orchestrated inflammatory response. The inflammatory response is initiated by the activation of pattern recognition receptors (PRRs) expressed by cells of the innate immune system, after binding with pathogen associated molecular patterns (PAMPs) associated with microbial pathogens.[61] The same system of receptors may be activated by endogenous molecules called alarmins, which are released from membranes of necrotic fetal cells and lead to sterile IAI.[11–13] Inflammation disrupts the matrix metalloproteinase (MMP)/ tissue-specific inhibitors of metalloproteinases (TIMPs) balance, resulting in collagenolysis and ultimately, weakens and ruptures the fetal membranes.[31]

There are several cytokines involved in intrauterine inflammation. Among proinflammatory cytokines, IL-6 is the most studied for a higher predictive value of chorioamnionitis and neonatal infection.[62] However its clinical usefulness has been limited by the large variability in thresholds used in different studies.[63] IL-6 is a pleotropic cytokine that has been shown to be an efficient marker of MIAC and intraamniotic inflammation.[24–28,44–46] Given what we know about the involvement of IL-6 in both physiologic and pathologic parturition, there is biologic plausibility for its use as a diagnostic test in the detection of intrauterine inflammation and/or infection. A recent study by Seliger et al. sampled IL-6 daily.[39] In women with PPROM and signs of fetal inflammation, Seliger et al. demonstrated a significant elevation in cervicovaginal IL-6 starting 48 hours prior to delivery when compared to controls.[39] Ultimately they posed that 48 hour notice could provide an advantage in determining delivery timing and reducing inflammation and/or infection-related neonatal complications.[39] Similarly, after measuring daily vaginal IL-6

concentrations in women with PPROM, Kunze et. al found higher levels of IL-6 48 hrs prior to delivery, in women with adverse outcomes.[57]

Our meta-analysis explored the diagnostic test accuracy of cervicovaginal IL-6 using IAI, MIAC, HCA, FIRS, funisitis, and EONS as our reference tests. Though we used several reference tests, all of them act as surrogates for intrauterine inflammation and/or infection. As a result, we were able to combine the findings from different studies to estimate a DOR. Our study found that cervicovaginal IL-6 had a DOR of 10.74 (95% CI 5.45, 21.17) in the detection of intrauterine inflammation/infection. A DOR above 10 indicates very good diagnostic abilities. [64] Based on our results, cervicovaginal IL-6 seems to have a good diagnostic accuracy in the detection of intraamniotic inflammation/infection in women with PPROM. This is further supported by an AUC of 0.838 ± 0.035.

Based on the assessment of Cochrane's Q there is a large amount of heterogeneity in the DORs of each study. Gestational age is a biologically plausible source of heterogeneity. Seliger et al demonstrated overall higher accuracy for IL-6 at earlier gestational ages.[39] Around delivery, leukocyte infiltration increases inflammatory outputs, which propagate through the gestational tissues and into the fetal compartment, inducing further leukocyte infiltration; IL-6 is among the most generated cytokines in parturition.[65] During pregnancy, elevated levels of cervical and amniotic fluid IL-6 have been linked to the process of cervical ripening and the impending onset of labor[38,66,67] We therefore decided to use gestational age as a covariate in our global assessment of the accuracy of cervicovaginal IL-6 as a diagnostic test for intrauterine inflammation/infection. Interestingly, cervicovaginal IL-6 was noted to have a high sensitivity at

GA < 34 weeks and a high specificity at GA <37 weeks in the detection of intraamniotic inflammation (figures 6a & 6b).

Other possible sources of heterogeneity include different sampling techniques, method of IL-6 quantification and timing of IL-6 sampling in relation to reference test sampling. The majority of studies used cervicovaginal swabs collected on speculum exam. However alternate methods, including aspiration (via syringes and monovettes), fluid collection from sanitary pads through the use of a garlic press, and the use of novel devices (Yoon's AF Collector^{TM}; novel cervical fluid collectors) were also implemented. The immunoassays were all conducted with different kits. Furthermore, studies were conducted over a 23-year period (1998-2021) with a likely improvement in the accuracy of the qualitative and quantitative tests used in the detection of IL-6 adding thus another variable/possible source of non-random heterogeneity. Methods of collection and quantification (various forms of immunoassays) could not be explored as a covariate given the limited number of studies utilizing each method. Finally, the temporal relationship between assessment of cervicovaginal IL-6 concentration and the interpretation of the reference test, was uncertain in several studies. The uncertainty in the temporal relationship between index test and reference test assessment introduces heterogeneity and makes the relationship difficult to interpret. Inflammation and/or infection could be present at delivery, but if absent at the time of diagnosis when IL-6 levels were assessed, an accurate depiction of the intrauterine milieu at delivery would not be provided by the cervicovaginal IL-6 concentration due to the varying timepoints of assessment. The exception to this concern was the use of IAI or MIAC as reference tests since the sample of amniotic fluid used for the assessment of IAI and/or MIAC is collected and interpreted at the same time as the cervicovaginal sample of IL-6.

2.5 Strengths and Limitations

There are several strengths to our study. Our review of the literature was extensive, and our search was conducted in a systematic and reproducible manner. To limit reporting bias, the study protocol was prospectively designed and registered at the international prospective register of systematic reviews. Finally, by considering biologic plausibility in our analysis, our interpretation has the potential for more robust clinical applications.

There were some limitations to our study. Like any systematic review and meta-analysis, our study was dependent on the quality of the individual studies evaluated. Based on our QUADAS-2 assessment, there was a high level of bias in at least one category for the majority of our studies. By nature, diagnostic studies often have heterogeneous thresholds for index test results making the determination of a summary point (a specific threshold with an aggregated sensitivity and specificity) inappropriate. Instead, a SROC must be used to aggregate the data which may be more difficult to apply clinically. Another limitation is our use of surrogate reference standards in the diagnosis of intrauterine inflammation/infection due to the lack of a gold standard. Finally, heterogeneity in the vaginal microbiome between different races has been demonstrated.[68] A predominance of non-lactobacilli, seen in higher proportion in certain race and ethnic groups, is known to increase the cervical inflammatory response and can therefore influence the heterogeneity present in our study.[69] Unfortunately, many of the studies did not report maternal race thus we could not account for the effect of race on our results. In addition to adding to the heterogeneity, the lack of information on race makes it difficult to assess the generalizability of our findings.

2.6 Conclusions and Implications

Based on our results, cervicovaginal IL-6 appears to have a high accuracy in the detection of intrauterine inflammation and/or infection. It is a noninvasive to minimally invasive test (depending on the method of collection used) that could be utilized to monitor the development of intraamniotic inflammation and/or infection in women diagnosed with PPROM (repeated sampling would be possible). It also has the potential to decrease the number of amniocenteses necessary to diagnose intrauterine inflammation/infection. For example, Supplemental figure 1a depicts IAI as the reference test. It demonstrates a specificity of 0.91 [95% CI 0.83-0.96] for the two studies. Though there were only two studies looking at this, we can infer that high levels of cervicovaginal IL-6 correlate with an inflammatory intrauterine environment. Both studies (Kacerovsky 2018 and Musilova 2016) collected cervicovaginal IL-6 and amniotic fluid at the time of PPROM diagnosis and used lateral flow immunoassay on fresh samples to determine the concentration of cervicovaginal IL-6 with 2500pg/mL as their threshold. Based on the two studies looking at IAI, the median prevalence is 14% with an interquartile range of 12% to 17%. This is consistent with a prevalence of 4% to 29% seen in the literature.[70] In a group of 1000 women, using 14% as the prevalence (140 women with IAI and 860 without) and 91% as the specificity, 77 women would have a false positive test and thus undergo an amnio unnecessarily. Given the low risk of complications associated with amniocentesis, 91% specificity seems reasonable if cervicovaginal IL-6 is used as a screening test to determine which women with PPROM should undergo amniocentesis to rule in inflammation/infection.

Our finding of increased sensitivity at less than 34 weeks and increased specificity in groups including women that are further along in GA are relevant when considering the recent changes in recommended delivery timing of women with PPROM. Cervicovaginal IL-6 could potentially provide qualitative evidence to aid in management considerations at 34 weeks (continue expectant management or proceed with delivery). Finally, a bedside point-of-care test for the detection of IL-6 exists and has been used experimentally in Europe, Asia, and Africa. The bedside test provides IL-6 results in 20 minutes. The test used in the study by Eleje et al in Nigeria had very high sensitivity (100%), and specificity (91%).[53] A prospective study, published after our analysis was conducted, used the same test to assess for the presence of HCA.[71] Their study included 110 women with PPROM and found that the semi-quantitative cervicovaginal IL6 bedside test had a sensitivity of 98.6%, specificity of 97.3%, PPV 97.3%, and NPV of 97.3%.[71] The rapid availability of results with the high sensitivity and specificity suggest a high possibility of clinical utility though additional studies are needed for validation.

As discussed previously, our findings are limited by the quality of the reviewed studies and the interstudy heterogeneity. Our findings alone are not robust enough to justify the implementation of cervicovaginal IL-6 in the management of PPROM. However, this study underscores the need for prospective studies to further evaluate the diagnostic utility of cervicovaginal IL-6.

3. DISCUSSION

3.1.a Application of Findings

The findings of our systematic review/meta-analysis support the need for prospective studies further investigating the diagnostic power of cervicovaginal IL-6. We have designed a prospective study that will assess the relationship between cervicovaginal IL-6 and associated morbidities in women diagnosed with PPROM prior to 34 weeks. We chose 34 weeks as the GA cutoff because based on the data, this population is where we anticipate the biggest impact of the cervicovaginal IL-6 diagnostic capabilities.

We plan to collect samples at the time that labor starts or at the time the decision to deliver is made in addition to the sample collected at diagnosis. This will give us info that is at an adequate time interval from the reference and will allow us to compare samples to assess the change in cervicovaginal IL-6 over time.

3.1.b Prospective Study

IL-6 concentrations analyzed from vaginal fluid have been shown to have a high negative predictive value for the detection of microbial-associated intraamniotic inflammation, intraamniotic inflammation, and microbial invasion of the amniotic cavity.[1] In addition to IL-6, various other acute phase cytokines have biological plausibility as predictors of intraamniotic inflammation. In a study by Laniewski et. al, IL-1a, IL-1b, IL-8, MIP-1B, CCL20(MIP-3a), RANTES, and TNFa, were used to develop a genital inflammation score.[72] A score of 5 out of 7 denoted genital inflammation.[72] We plan to use a multiplex analysis to examine vaginal fluid samples for various inflammatory markers to estimate a genital inflammation score.

Hypothesis: Following PPROM, vaginal IL-6 concentrations exceeding the inflammation threshold of 2.5ng/mL[1] are associated with a shorter latency vs IL-6 concentrations less than or equal to 2.5ng/mL.

Aims:

Primary: To determine the relationship between latency and vaginal fluid IL-6 concentration in women with PPROM.

Secondary:

- To determine the relationship between IL-6 concentrations and composite neonatal and maternal outcomes.
- To determine if there is an association between other inflammatory markers, the genital inflammation score, and latency or neonatal or maternal morbidity using a multiplex analysis.

3.1.c Project impact and outcomes:

We are proposing a prospective cohort study that will stratify women into two groups utilizing vaginal fluid collection for IL-6 analysis. Fluid will also be analyzed for other markers of inflammation and the results will be used to assign a genital inflammation score as previously described.[72] Following assessment of vaginal IL-6 concentration, and hospital admission per PPROM standard of care, subjects will be continuously monitored for the duration of their

hospitalization. Data obtained will be used to ascertain the relationship between IL-6 concentration, genital inflammation score, and pregnancy latency.

Prognostic data related to latency has profound implications. While timing is critical at all gestations, it is most critical for women with PPROM in the periviable (delivery between 20w0d and 25w6d gestation) period. PPROM at periviability significantly elevates the risk of adverse neonatal outcomes such as extremely preterm birth (<28 weeks of gestation), disability, and death.[73] In addition to these risks, periviable PPROM poses a unique management challenge due to the lack of concrete guidelines regarding the timing and implementation of preterm interventions. The interventions available include corticosteroids to promote fetal lung maturity, magnesium sulfate for fetal neuroprotection, latency antibiotics to help decrease neonatal morbidity and increase latency, and maternal hospitalization through the duration of pregnancy.[2] The American College of Obstetrics and Gynecology (ACOG) recommends implementation of all the aforementioned interventions at 24 weeks of gestation but states some may be considered at earlier gestations.[74]

3.1.d Final thoughts

A major issue in our systematic review and meta-analysis was heterogeneity. In our prospective study, sample collection and processing will be standardized. Additionally, we are collecting data on many confounders including gestational age on admission and delivery, maternal characteristics (age, BMI, race, medical history), and neonatal characteristics (apgars, cord blood gases, weight at delivery, data on clinical course) amongst others. Our hope is to limit the

amount of non-random variability to produce reliable data regarding the diagnostic accuracy of cervicovaginal IL-6 for intrauterine inflammation and/or infection in the setting of PPROM.

4. TABLES AND FIGURES

Figure 1: Study flow diagram

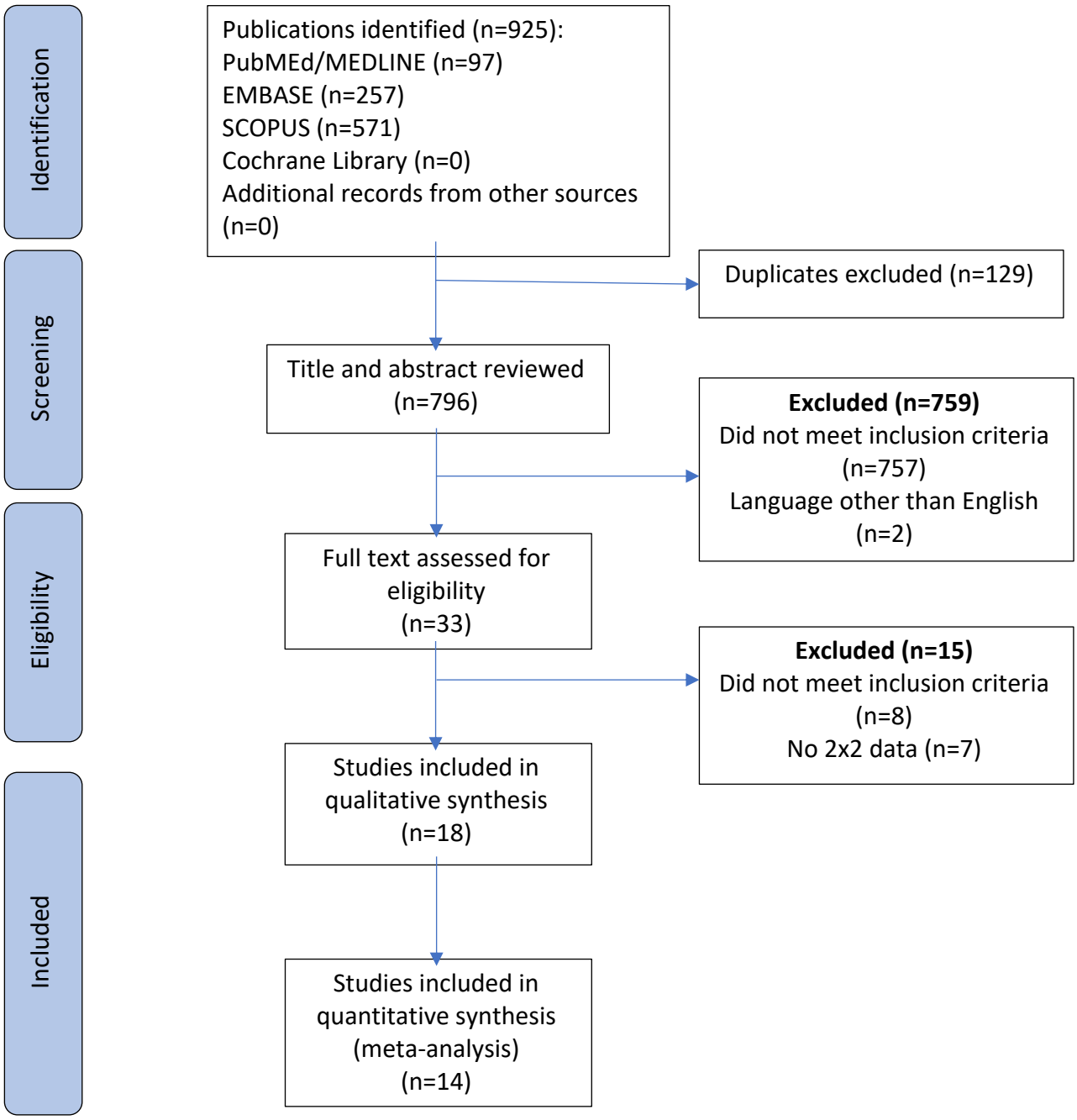


Table 1: Characteristics of studies included in the meta-analysis. *IGFBP + AFP

Reference	Country	Time of enrollment	Study design	No of participants (n)	Diagnosis of pprom	GA	Clinical management of PPROM			Index test(s)	Reference standard	Study prevalence %
							Antibiotics	Steroids	Tocolysis			
Seliger et al 2021[39]	Germany	Feb 2016-Jan 2018	Multicenter, Prospective Case-control	37	Pooling on speculum exam, Amnisure	24w0d-34w0d	Yes	Yes	Yes	Vaginal [IL-6]	HCA EONS FIRS	HCA 14/37: 38% EONS 7/37: 19%
Balciuniene et al 2021[33]	Lithuania	July 2017-July 2019	Single center Prospective Case-control	156	Pooling on speculum exam, Amnisure	22w0d-34w6d	Yes	Yes	NR	Vaginal [IL-6]	HCA	HCA 65/156: 42%
Eleje et al. 2020[53]	Nigeria	Aug 2017-Oct 2018	Multicenter, Prospective Case-control	48	Amnioquick duo*	24w0d-36w6d	Yes	Yes	Yes	Cervicovaginal [IL-6]	HCA	HCA 25/48: 52%
Mikolajczyk et al. 2020[36]	Poland	May 2013-Dec 2016	Single center, Prospective Cohort	80	History, AFI, pooling on speculum exam, Amnisure	24w0d-34w0d	Yes	Yes	Yes	Cervicovaginal [IL-6]	FIRS	FIRS 45/80: 56%
Musilova et. al 2017[51]	Czech Republic	Feb 2014-May 2016	Single center, Prospective cohort	144	Pooling on speculum, Actim Prom	24w0d-36w6d	Yes	Yes	Yes	Cervical [IL-6]	MIAC & IAI	MIAC + IAI: 19/144: 13%
Kayem et al. 2017[54]	France	Jan 2004-Feb 2006	Multicenter, Prospective cohort	184	History, speculum exam (not specified)	24w0d-36w6d	NR	NR	NR	Vaginal [IL-6]	EONS & HCA	EONS: 29/184: 15.8% HCA: 86/141:61%
Musilova et al. 2016[1]	Czech Republic	Aug 2014-March 2016	Single center, Prospective cohort	141	Pooling on speculum, Actim Prom	24w0d-36w6d	Yes	Yes	Yes	Vaginal [IL-6]	MIAC, IAI, MIAC +IAI	MIAC: 36/141: 26% IAI: 27/141: 19% MIAC + IAI: 17/141: 12%

Reference	Country	Time of enrollment	Study design	No of participants (n)	Diagnosis of pprom	GA	Clinical management of PPROM			Index test(s)	Reference standard	Study prevalence %
							Antibiotics	Steroids	Tocolysis			
Kacerovsky et al. 2014[52]	Czech Republic	Jan 2012-Nov 2013	Single center, Prospective cohort	68	Pooling on speculum, Actim Prom	24w0d-36w6d	Yes	Yes	Yes	Vaginal [IL-6]	MIAC & HCA	MIAC + HCA: 17/68: 25%
Kacerovsky et al. 2014[46]	Czech Republic	Jan 2012-July 2013	Single center, Prospective cohort	60	Pooling on speculum, Actim Prom	24w0d-36w6d	Yes	Yes	Yes	Cervical [IL-6]	MIAC & HCA	MIAC + HCA: 12/60: 20%
Ryu et al. 2013[24]	South Korea	Nov 2008-Oct 2011	Single center, Prospective cohort	76	Pooling and nitrazine on scpeculum	20w0d-35w0d	Yes	Yes	Yes	Cervicovaginal [IL-6]	MIAC	MIAC: 35/76 46%
Lucovnik et al. 2011[55]	Slovenia	April 2007-Feb 2009	Single center, Prospective cohort	42	Pooling on speculum, Actim Prom	23w6d-31w6d	Yes	Yes	NR	Vaginal [IL-6]	HCA	HCA: 29/42: 69%
Torbe et a. 2006[59]	Poland	NR	Single center, Prospective cohort	62	Pooling and nitrazine on speculum	24w0d-34w0d	Yes	Yes	NR	Vaginal [IL-6]	EONS	EONS: 21/62: 34%
Kayem et al. 2005[42]	France	Jan 2000-Dec 2001	Single center, Prospective cohort	73	NR	24w0d-34w0d	NR	NR	NR	Vaginal [IL-6]	EONS	EONS: 14/73 (19%)
Jun et al. 2000[44]	South Korea	NR	Single center, Prospective cohort	86	Pooling, nitrazine, ferning on speculum	<36w0d	NR	NR	NR	Cervical [IL-6]	MIAC	MIAC: 12/86: 14%
Rizzo et al. 1998[45]	Italy	Jan 1993-Aug 1997	Single center, Prospective cohort	124	Pooling, nitrazine, ferning on speculum, AFI	24w0d-32w0d	Yes	Yes	Yes	Cervical [IL-6]	MIAC	MIAC: 42/124: 33.8%
Abdelazim 2013[56]	unclear	NR	Unclear if Single or multicenter, Prospective cohort	120	Pooling, nitrazine, ferning on speculum, AFI, History	34w0d-37w0d	NR	NR	NR	Cervicovaginal [IL-6]	EONS HCA	EONS: 53/120: 44% HCA: 49/120: 40.8%

Reference	Country	Time of enrollment	Study design	No of participants (n)	Diagnosis of pprom	GA	Clinical management of PPROM			Index test(s)	Reference standard	Study prevalence %
							Antibiotics	Steroids	Tocolysis			
Matsuda et al. 2000[60]	Japan	NR	Single center, Prospective cohort	36	Pooling, nitrazine, ferning on speculum	<36wks	Yes	No	NR	Cervicovaginal [IL-6]	EONS	EONS: 9/36: 25%
Kacerovsky et al. 2018[50]	Czech Republic	Jan 2016-Feb 2017	Single center, Prospective cohort	118	Pooling on speculum, Actim PROM	34w0d-36w6d	Yes	NR	NR	Vaginal [IL-6]	IAI MIAC	IAI: 11/118 9% MIAC: 25/118 21%

Table 2: Characteristics of studies excluded with reasons

Reference	Country	Time of enrollment	Study design	No of participants (n)	Diagnosis of pprom	GA	Exclusion criteria	Reason for exclusion
Lee et al. 2018[58]	South Korea	Nov 2008-Jul 2014	Single center, Prospective cohort	75	Pooling, nitrazine, ferning on speculum	21w0d-34w0d	Multiples, major congenital anomalies, vaginal bleeding, prior cervical cerclage, active labor (presence of cervical dilation > 3cm by sterile speculum exam), clinical signs of chorioamnionitis	Not enough info to derive 2x2 table
Dorfeuille et al. 2016[75]	Canada	Jan 2013-Sept 2014	Single center, Prospective cohort	25	Ferning on speculum	22w0d-36w0d	Lethal fetal or chromosomal anomaly, clinical suspicion of PPROM for longer than 48 hrs, HIV, chronic hep B or C, active herpetic lesions, active labor, preeclampsia, maternal temperature > 38ºC, non-reassuring fetal status, age < 18	No cutoff value for IL-6 described and not enough information to derive 2x2 table
Kunze et al. 2016[57]	Germany	Jan 2010-Feb 2014	Multicenter, Prospective case-control	99	History, AFI, Pooling, nitrazine on speculum	23w0d-33w1d	No congenital anomalies, non-reassuring fetal status, active labor with cervical dilation ≥3cm, clinical chorioamnionitis, outborn neonates (excluded due to missing samples and neonatal data)	Not enough information to derive 2x2 table
Cobo et al. 2014[76]	Spain	Sept 2005-June 2008	Single center, Prospective cohort	33	Pooling, nitrazine on speculum	< 34w0d	Multiples, structural/chromosomal anomalies, clinical chorioamnionitis, vaginal bleeding at admission, amniocentesis not possible	No information regarding IL-6 values
Vousden et al. 2011[77]	United Kingdom	Mar 2008-2009	Single center, Prospective cohort	11	NR	NR	Indicated immediate delivery, non-reassuring fetal status, fetal malformation, active labor, sexual intercourse in the last 24h, fresh blood visible on speculum exam	Not enough information to derive 2x2 table
Holstrom et al 2019[38]	Finland	June 2013-July 2017	Single center, Prospective cohort	25	Actim Prom	22w0d-37w0d	Insulin-dependent diabetes, vaginal bleeding, fetal chromosomal abnormality	Not enough information to derive 2x2 table
Beneventi et al. 2016[78]	Italy	Jan 2012-Dec 2013	Single center, Prospective case-control	24	NR	<37wks	NR	Not enough information to derive 2x2 table

Figure 2a: Risk of bias summary of included studies

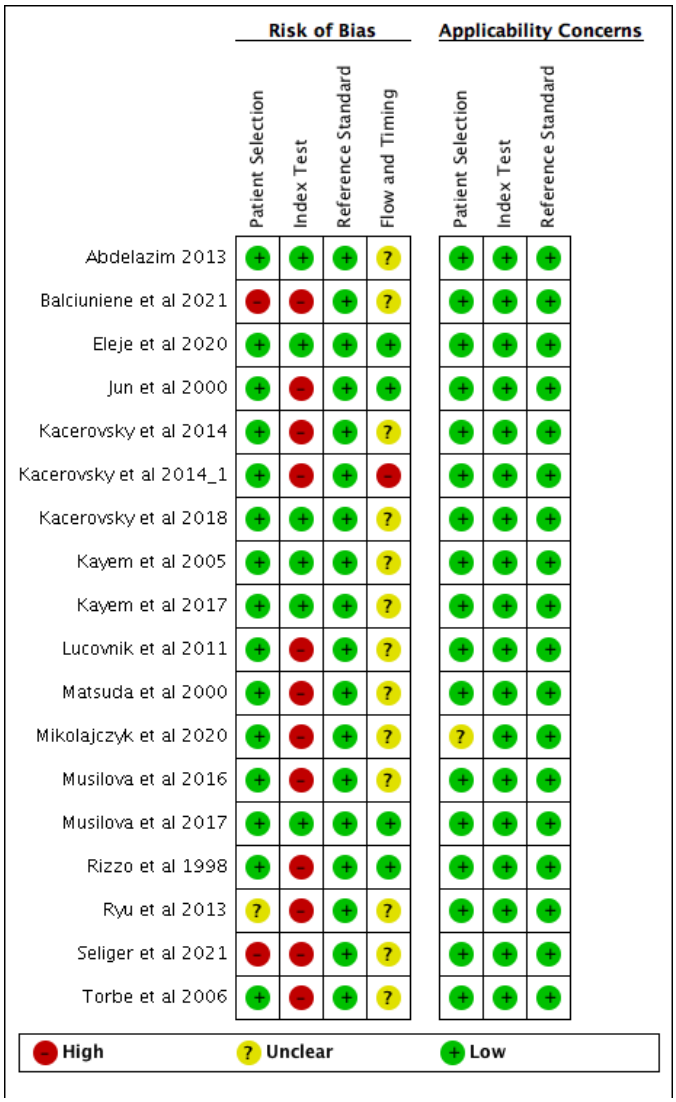
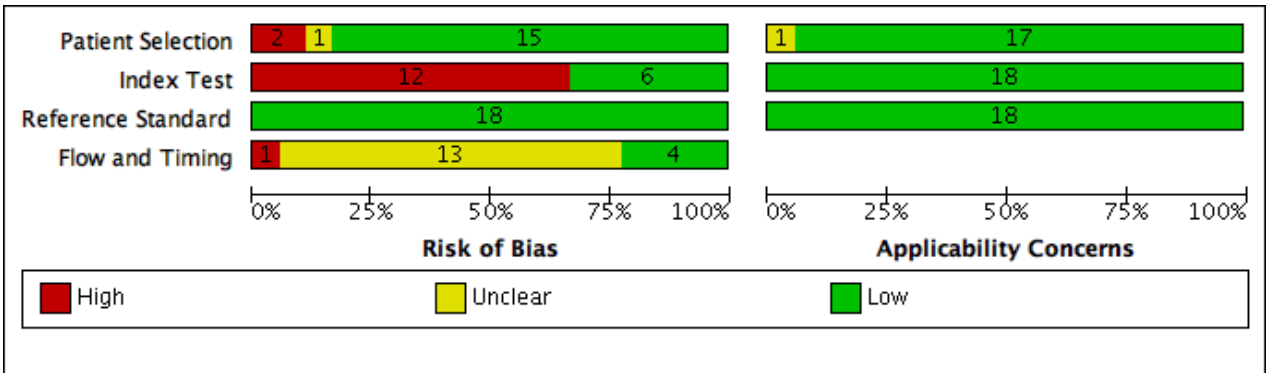
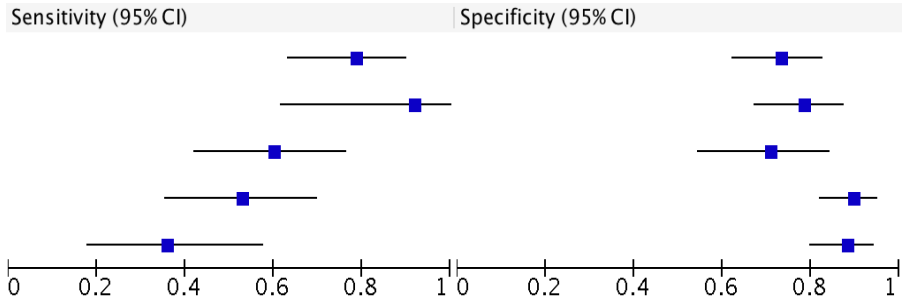


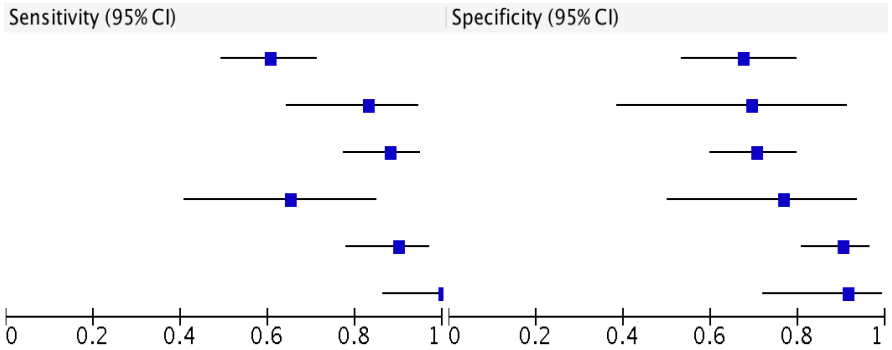
Figure 2b: Risk of bias graph of included studies



Study	TP	FP	FN	TN	[IL-6] pg/ml	SN (95% CI)	SP (95% CI)
Rizzo et al 1998*	33	22	9	60	200	0.79 [0.63, 0.90]	0.73 [0.62, 0.82]
Jun et al 2000^	11	16	1	58	350	0.92 [0.62, 1.00]	0.78 [0.67, 0.87]
Ryu et al 2013^	21	12	14	29	483	0.60 [0.42, 0.76]	0.71 [0.54, 0.84]
Musilova et al 2016^	19	11	17	93	2500	0.53 [0.35, 0.70]	0.89 [0.82, 0.95]
Kacerovsky et al 2018#	9	11	16	82	2500	0.36 [0.18, 0.57]	0.88 [0.80, 0.94]



Study	TP	FP	FN	TN	[IL-6] pg/ml	SN (95% CI)	SP (95% CI)
Kayem et al 2017^	52	18	34	37	50000	0.60 [0.49, 0.71]	0.67 [0.53, 0.79]
Lucovnik et al 2011*	24	4	5	9	1045	0.83 [0.64, 0.94]	0.69 [0.39, 0.91]
Balciuniene et al 2017*	57	27	8	64	1390	0.88 [0.77, 0.95]	0.70 [0.60, 0.79]
Seliger et al 2021*	13	4	7	13	6417	0.65 [0.41, 0.85]	0.76 [0.50, 0.93]
Abdelazim 2013#	44	7	5	64	100	0.90 [0.78, 0.97]	0.90 [0.81, 0.96]
Eleje et al 2020^	25	2	0	21	100	1.00 [0.86, 1.00]	0.91 [0.72, 0.99]



Study	TP	FP	FN	TN	[IL-6] pg/ml	SN (95% CI)	SP (95% CI)
Seliger et al 2021*	19	11	1	6	1150	0.95 [0.75, 1.00]	0.35 [0.14, 0.62]
Kayem et al 2017^	17	79	12	76	50000	0.59 [0.39, 0.76]	0.49 [0.41, 0.57]
Kayem et al 2005*	11	26	3	33	100	0.79 [0.49, 0.95]	0.56 [0.42, 0.69]
Abdelazim 2013#	42	8	11	59	100	0.79 [0.66, 0.89]	0.88 [0.78, 0.95]
Matsuda et al 2000^	9	3	0	24	70000	1.00 [0.66, 1.00]	0.89 [0.71, 0.98]
Torbe et al 2006*	7	3	14	38	2000	0.33 [0.15, 0.57]	0.93 [0.80, 0.98]

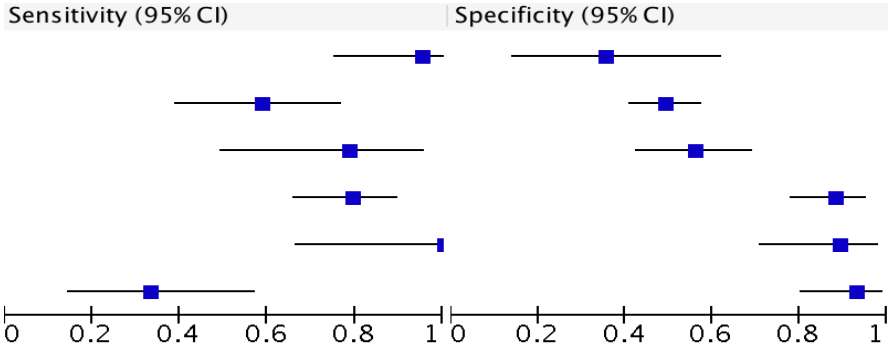


Figure 3a: MIAC, 3b: HCA, 3c: EONS
* < 34wks GA # >34wks GA ^ mixed GA

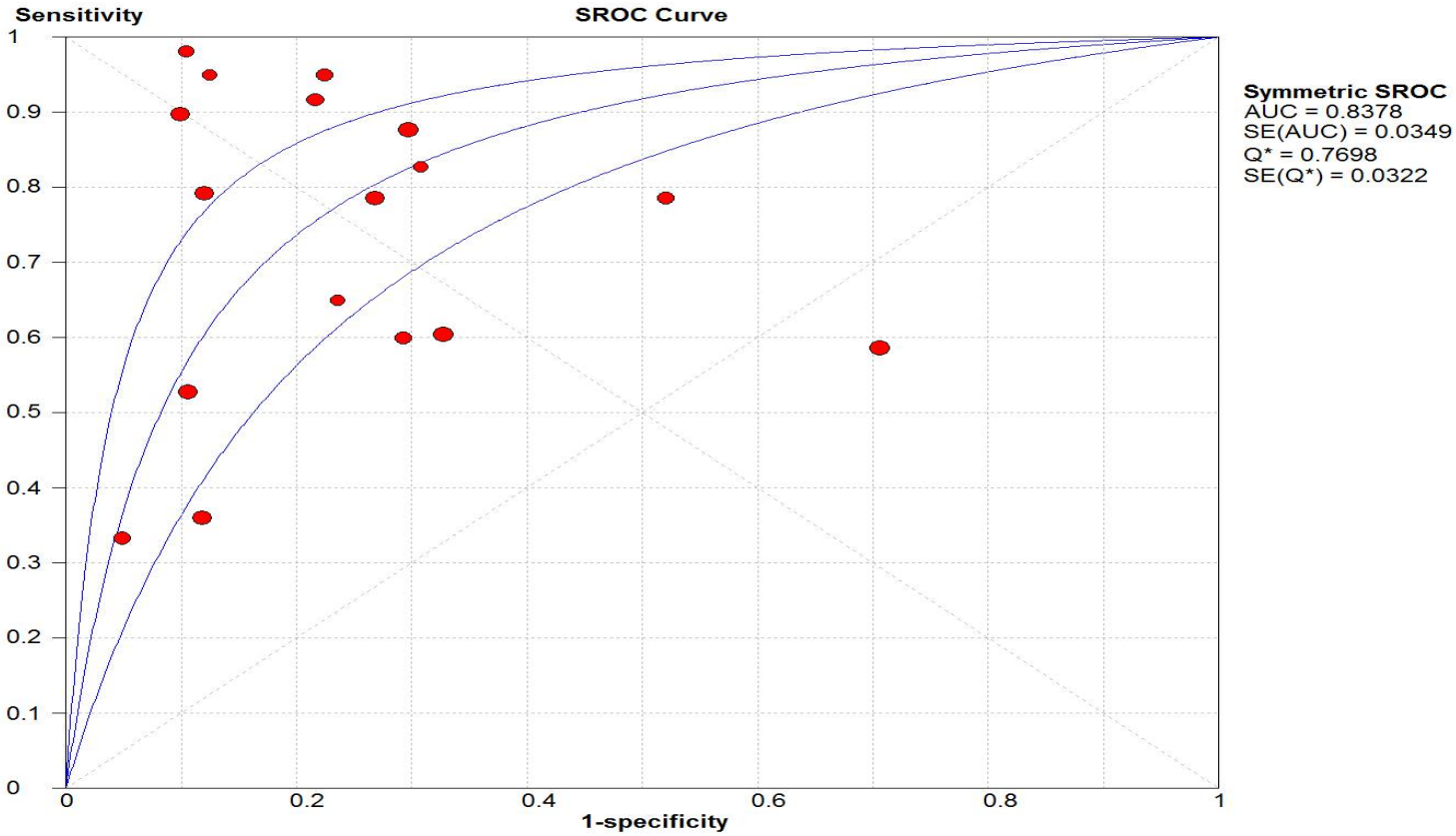


Figure 4: *SROC Plot for Included Studies*

Table 3

Study	*DOR*	95% *CI*	% Weight
Torbe et al. (2006)	9.833	2.255- 42.882	5.76
Kayem et al. (2005)	3.385	0.841 - 13.615	5.94
Abdelazim (2013a)	28.159	10.432 - 76.008	6.76
Matsuda et al. (2000)	133.00	6.258 - 2826.4	3.04
Seliger et al. (2021a)	6.036	1.417 - 25.710	5.81
Balciuniene et al. (2021)	16.889	7.104 - 40.150	7.01
Eleje et al. (2020)	438.60	19.955 - 9640.400	3.00
Kayem et al. (2017a)	3.144	1.546 - 6.394	7.28
Lucovnik et al. (2011)	10.800	2.358 - 49.464	5.66
Musilova et al. (2016)	9.449	3.823 - 23.353	6.93
Ryu et al. (2013)	3.625	1.396 - 9.410	6.84
Jun et al. (2000)	39.875	4.783 - 332.41	4.46
Rizzo et al. (1998)	10.000	4.130 - 24.211	6.97
Kacerovsky et al. (2018)	4.193	1.495 - 11.757	6.69
Seliger et al. (2021b)	65.636	7.879 - 546.77	4.47
Kayem et al. (2017b)	0.592	0.255 - 1.375	7.05
Abdelazim (2013b)	80.457	23.989- 269.85	6.32

Note. (REM) pooled DOR = 10.74 (*CI* = 5.445 - 21.168)
Heterogeneity x^2 = 88.03 (*df* = 16), *p* = .000
Inconsistency (I^2) = 81.8%
Estimate of between-study variance (Tau-squared) = 1.5169
Number of studies = 17.

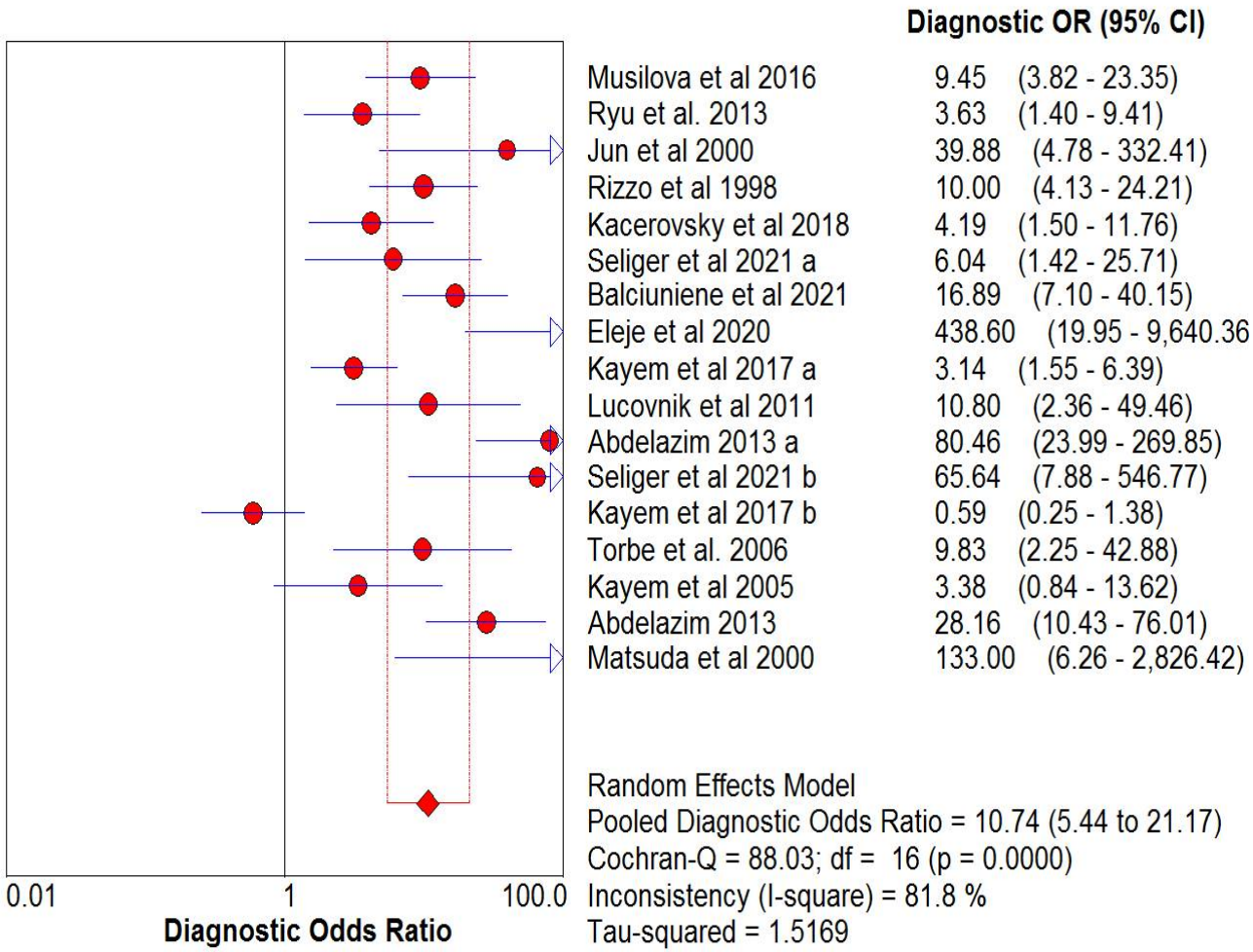
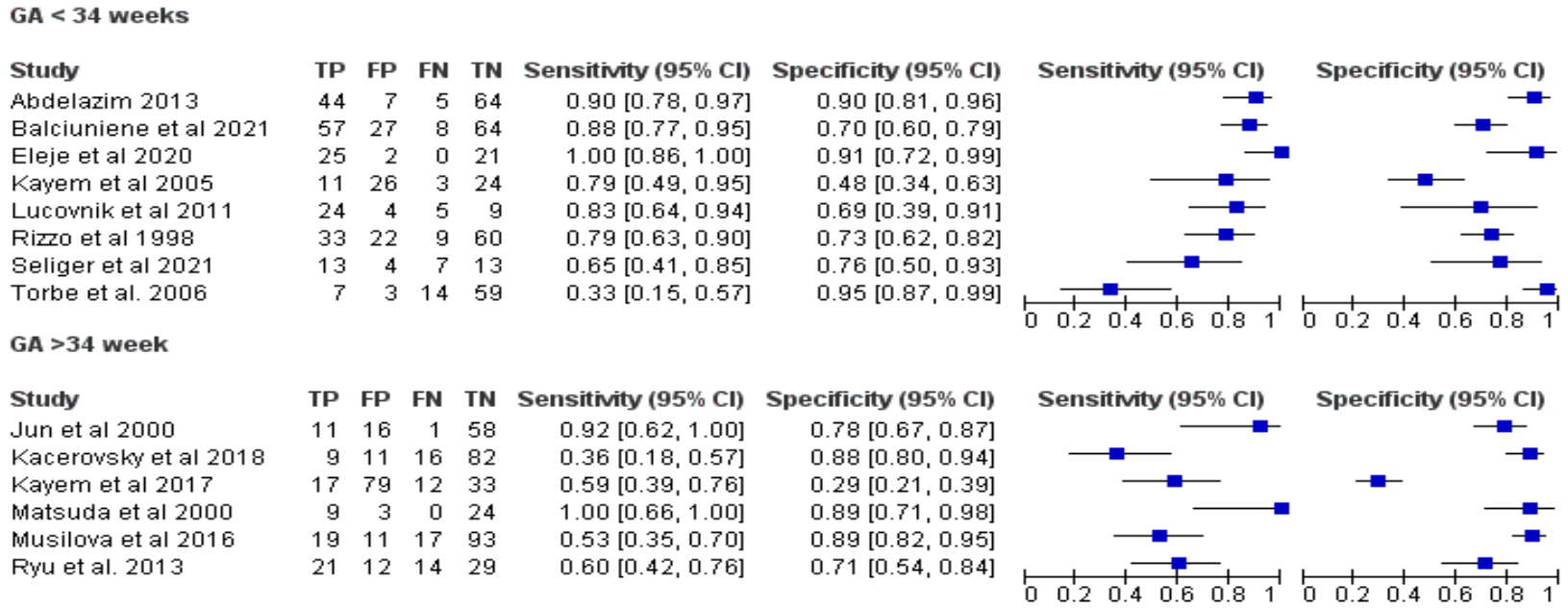


Figure 5: *Diagnostic Odds Ratio of IL-6 in the overall detection of intrauterine inflammation/infection*

Figure 6a

Forest Plot for the Overall Comparison of Studies for Women GA<34 weeks vs <37 weeks



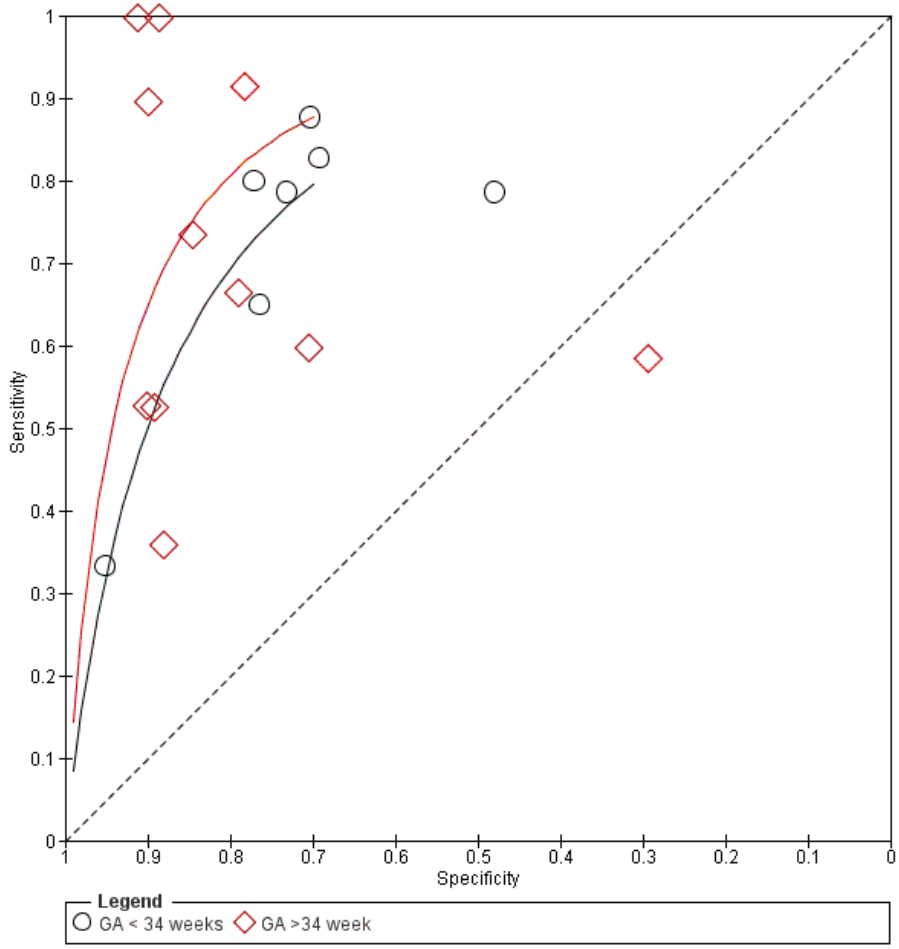


Figure 6b

SROC of Included Studies (GA < 34 Weeks Vs GA < 37)

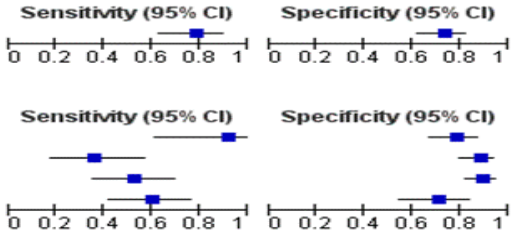
A

GA less than 34 week

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Rizzo et al 1998	33	22	9	60	0.79 [0.63, 0.90]	0.73 [0.62, 0.82]

GA greater than 34 weeks

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Jun et al 2000	11	16	1	58	0.92 [0.62, 1.00]	0.78 [0.67, 0.87]
Kacerovsky et al 2018	9	11	16	82	0.36 [0.18, 0.57]	0.88 [0.80, 0.94]
Musilova et al 2016	19	11	17	93	0.53 [0.35, 0.70]	0.89 [0.82, 0.95]
Ryu et al. 2013	21	12	14	29	0.60 [0.42, 0.76]	0.71 [0.54, 0.84]



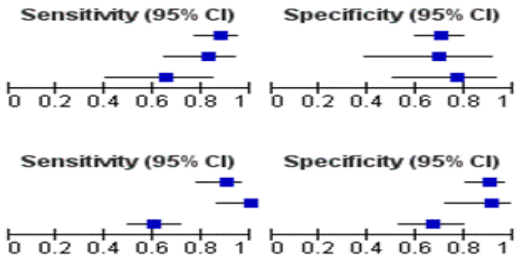
B

GA less than 34

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Balciuniene et al 2021	57	27	8	64	0.88 [0.77, 0.95]	0.70 [0.60, 0.79]
Lucovnik et al 2011	24	4	5	9	0.83 [0.64, 0.94]	0.69 [0.39, 0.91]
Seliger et al 2021	13	4	7	13	0.65 [0.41, 0.85]	0.76 [0.50, 0.93]

GA greater than 34

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Abdelazim 2013	44	7	5	64	0.90 [0.78, 0.97]	0.90 [0.81, 0.96]
Eleje et al 2020	25	2	0	21	1.00 [0.86, 1.00]	0.91 [0.72, 0.99]
Kayem et al 2017	52	18	34	37	0.60 [0.49, 0.71]	0.67 [0.53, 0.79]



C

GA less than 34

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Kayem et al 2005	11	26	3	24	0.79 [0.49, 0.95]	0.48 [0.34, 0.63]
Seliger et al 2021	19	11	1	38	0.95 [0.75, 1.00]	0.78 [0.63, 0.88]
Torbe et al. 2006	7	3	14	59	0.33 [0.15, 0.57]	0.95 [0.87, 0.99]

GA greater than 34

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Abdelazim 2013	42	8	11	59	0.79 [0.66, 0.89]	0.88 [0.78, 0.95]
Kayem et al 2017	17	79	12	33	0.59 [0.39, 0.76]	0.29 [0.21, 0.39]
Matsuda et al 2000	9	3	0	24	1.00 [0.66, 1.00]	0.89 [0.71, 0.98]

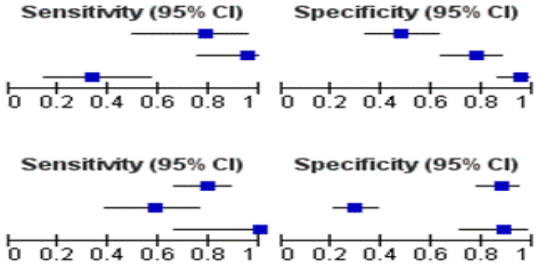
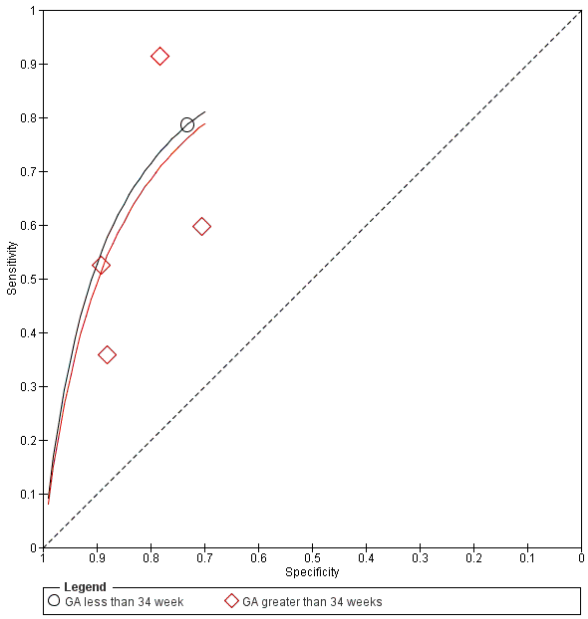
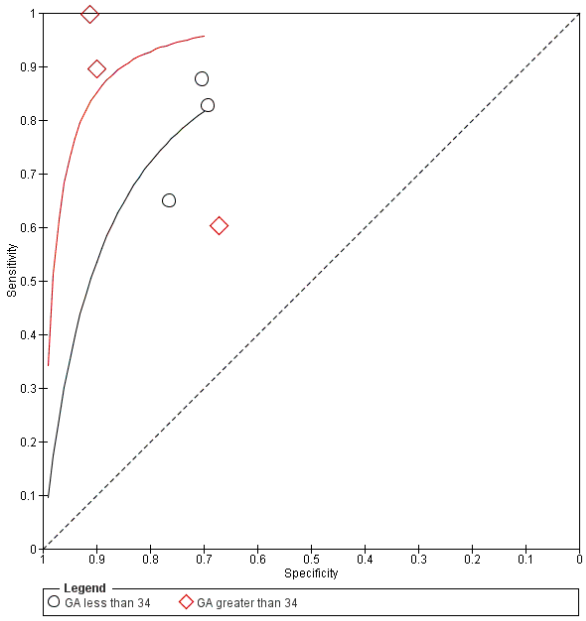


Figure 7a, 7b, & 7c

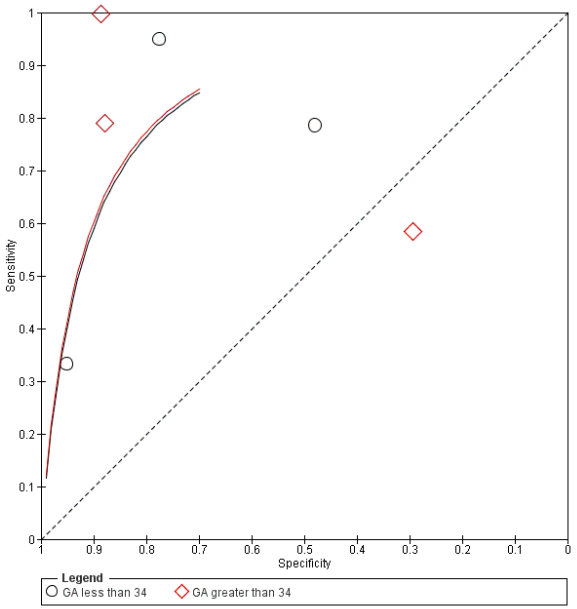
Forest Plot of Sensitivity, Specificity, and Variability for GA < 34 vs <37 wks GA for MIAC (A), HCA (B), and EONS (C)



A



B



C

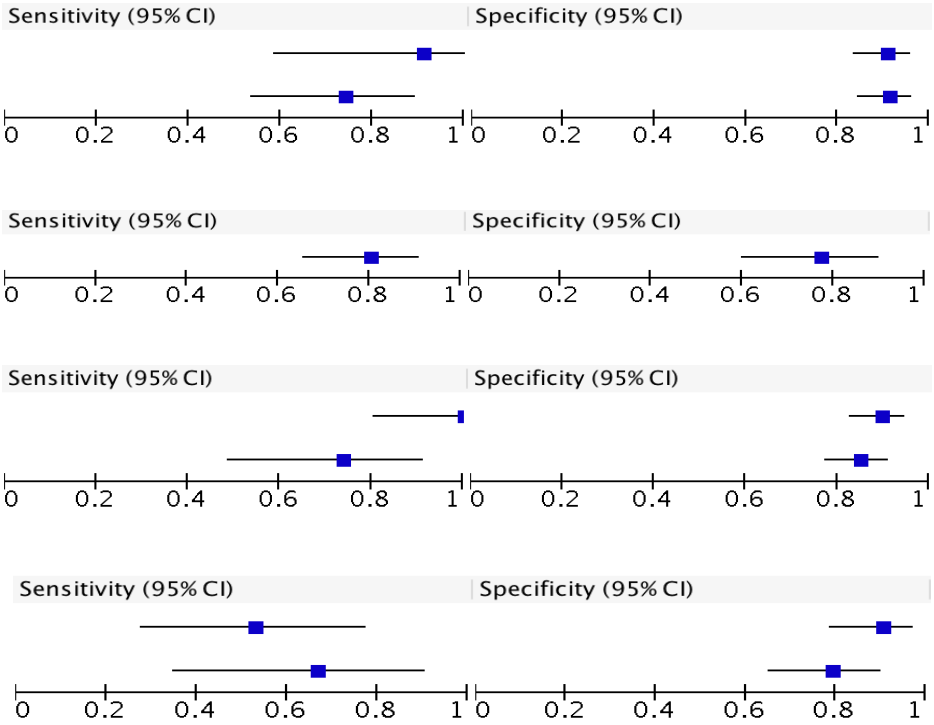
Figure 8a, 8b, 8c
SROC curves for GA < 34wks vs. GA <37wks for MIAC (A), HCA (B), and EONS (C)

Study	TP	FP	FN	TN	[IL-6] pg/ml	SN (95% CI)	SP (95% CI)
Kacerovsky et al 2018#	10	10	1	97	2500	0.91 [0.59, 1.00]	0.91 [0.83, 0.95]
Musilova et al 2016^	20	10	7	104	2500	0.74 [0.54, 0.89]	0.91 [0.84, 0.96]

Study	TP	FP	FN	TN	[IL-6] pg/ml	SN (95% CI)	SP (95% CI)
Mikolajczy et al 2020*	36	8	9	27	27	0.80 [0.65, 0.90]	0771 [0.60, 0.90]

Study	TP	FP	FN	TN	[IL-6] pg/ml	SN (95% CI)	SP (95% CI)
Musilova et al 2016^	17	13	0	111	2500	1.00 [0.80, 1.00]	0.90 [0.83, 0.94]
Musilova et al 2017^	14	19	5	106	500	0.74 [0.49, 0.91]	0.85 [0.77, 0.91]

Study	TP	FP	FN	TN	[IL-6] pg/ml	SN (95% CI)	SP (95% CI)
Kacerovsky et al 2014^	9	5	8	46	462	0.53 [0.28, 0.77]	0.90 [0.79, 0.97]
Musilova et al 2014_1^	8	10	4	38	349	0.67 [0.35, 0.90]	0.79 [0.65, 0.90]



Supplemental Figure 1a: IAI 1b: FIRS 1c: MIAC + IAI 1d: MIAC + HCA
* < 34wks GA # >34wks GA ^ mixed GA

Supplemental table 1: Search strategy	
Query syntax:	((PPROM OR preterm prelabor rupture of membranes OR preterm premature rupture of membranes OR preterm prelabour rupture of membranes) AND (vagina* OR cervi*) AND (IL-6 OR IL6 OR IL 6 OR interleukin-6 OR interleukin 6))
Inclusion criteria:	▪ Diagnosis of preterm prelabor rupture of membranes (PPROM) ▪ Cervicovaginal interleukin-6 (IL-6) sample collected ▪ One or more reference standards assessed: Intraamniotic infection (IAI), microbial invasion of the amniotic cavity (MIAC), histologic chorioamnionitis (HCA), fetal inflammatory response syndrome (FIRS), funisitis, early onset neonatal sepsis (EONS) ▪ Gestational age less than 37 weeks ▪ 2x2 data available
Exclusion criteria:	▪ No PPROM ▪ No cervicovaginal assessment of IL-6 ▪ No assessment of any of the prespecified reference standards (IAI, MIAC, HCA, FIRS, funisitis, or EONS) ▪ No 2x2 data available ▪ Review, conference abstract ▪ Written in language other than English
Protocol	☐ Query syntax used to search databases: Embase, SCOPUS, PubMed, and the Cochrane library from their inception to Feb 2022 ☐ References of relevant publications were manually searched for additional potentially relevant published studies. ☐ Returned results were searched for duplicates and the duplicates were eliminated ☐ Remaining article titles and abstracts were screened for inclusion/exclusion criteria by reviewer D.G. and separately confirmed by reviewer C.C ☐ Full text articles were separately assessed for inclusion/exclusion criteria by D.G & C.C; disagreements settled with discussion and third reviewer, K.G. ☐ Data sheet piloted on 3 randomly chosen studies ☐ Two reviewers (D.G & C.C) separately pulled data from included articles; disputes were settled through discussion and by a third reviewer, K.G.

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