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Role of serum biomarkers in the prediction of outcome in women with threatened miscarriage: a systematic review and diagnostic accuracy meta-analysis

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BACKGROUND: Threatened miscarriage affects one in five women and is associated with significant emotional distress. The uncertainty around the prognosis of threatened miscarriage makes it equally challenging to the healthcare professionals. Various biochemical markers have been investigated in the past to predict the outcome of threatened miscarriage; however, the results have been conflicting. Therefore, we have conducted a systematic review and meta-analysis to determine the diagnostic accuracy of biochemical markers in predicting the outcome in women presenting with threatened miscarriage.

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METHODS: This is a systematic review and meta-analysis of prospective studies that investigated biochemical markers to determine outcomes for women with threatened miscarriage at 5–23 weeks gestational age. Electronic databases were searched up to June 2015 and study quality assessment was performed using QUADAS-2 (Quality Assessment for Diagnostic Accuracy Studies-2: A Revised Tool) for evaluating the diagnostic accuracy studies. Statistical analysis was performed using the Cochrane systematic review software.

RESULTS: A total of 19 studies were included in the qualitative data synthesis of which 15 (including 1263 women) were eligible for the meta-analysis. The review highlights the role of biochemical markers serum progesterone, hCG, pregnancy associated plasma protein A, estradiol and cancer antigen 125 (CA 125) in the prediction of outcome in women with threatened miscarriage. Interestingly, serum CA 125 appears to be the most promising marker ($n = 648$ women in seven studies), whereas serum progesterone and hCG are less useful once fetal viability is established. The summary receiver operating characteristics for CA 125 showed a sensitivity of 90% (95% confidence interval (CI) 83–94%), specificity of 88% (95% CI 79–93%), positive likelihood ratio of 7.86 (95% CI 4.23–14.60) and negative likelihood ratio of 0.10 (95% CI 0.06–0.20). The inverse of negative likelihood ratio was 9.31 (95% CI 5–17.1) indicating that a negative test is likely to identify those who are likely to continue with the pregnancy. Serum estradiol was the next best marker with a sensitivity of 45% (95% CI 6–90%), a specificity of 87% (95% CI 81–92%), a positive likelihood ratio of 3.72 (95% CI 1.01–13.71) and a negative likelihood ratio of 0.62 (95% CI 0.20–1.84).

CONCLUSIONS: In women with threatened miscarriage, serum CA 125 has high predictive value in identifying pregnancies that are 'likely to continue', whereas the most commonly used biomarkers of serum hCG and progesterone are not useful in predicting outcome of a pregnancy with a viable fetus. Other markers such as inhibin A and a combination of markers need to be investigated to hopefully improve the prediction of outcome in women with threatened miscarriage.

Key words: miscarriage / biomarkers / threatened / meta-analysis / outcome / cancer antigen 125 / hCG / serum progesterone / serum estradiol

Introduction

Miscarriage is the most common early pregnancy complication affecting 20% of recognized pregnancies (Savitz *et al.*, 2002; NICE Guideline CG 154, 2012). Threatened miscarriage is diagnosed when the woman presents in early pregnancy with vaginal bleeding, a closed cervix on clinical examination and subsequent ultrasound scan (USS) demonstrates fetal cardiac activity (Saraswat *et al.*, 2010; NICE Guideline CG 154, 2012). It is reported to occur in about one-fifth of pregnancies (Everett, 1997) but an estimated 3–16% of these subsequently miscarry (Cashner *et al.*, 1987; Siddiqi *et al.*, 1988; Hill *et al.*, 1991; Makrydimas *et al.*, 2003). Women presenting with threatened miscarriage are often extremely distressed and providing care can be challenging to the health care professionals, more so since it is difficult to provide reasonable information on the potential outcome. These women end up with repeated scans in early pregnancy units to allay their anxieties, which in turn adds to the increase in waiting times and costs. In the presence of reliable predictive biomarkers, the above challenges can be mitigated and potentially new therapeutics can be directed at those identified at an increased risk of miscarriage.

Various biochemical markers have been studied to establish if they are able to predict the outcome of threatened miscarriage (i.e. identify those at risk of subsequent miscarriage), however results have been conflicting. Some of the commonly studied biochemical markers are serum hCG, progesterone, estradiol, pregnancy associated plasma protein A (PAPP-A), cancer antigen 125 (CA 125), human placental lactogen (HPL), alpha feto-protein (AFP), inhibin A, follistatin and activin A (Westergaard *et al.*, 1985; Ruge *et al.*, 1990; Scarpellini *et al.*, 1995; Vavilis *et al.*, 2001; Johns *et al.*, 2007; Maged and Mostafa, 2013). In view of the conflicting evidence, we performed a systematic review and meta-analysis to determine which biochemical markers have high diagnostic accuracy to predict the outcome of threatened miscarriage either singly or in combination.

Materials and Methods

Study eligibility criteria

The inclusion criteria for the systematic review were all prospective studies with use of biochemical markers to determine outcomes for women with threatened miscarriage and gestational age between 5 and 23 weeks. Exclusion criteria were retrospective studies, case reports, case series, letters, and reviews; studies that did not include women in the period 5–23 weeks; studies with infertility, recurrent miscarriage or pregnancy of unknown location (PUL) cohorts or where women had ovulation induction medications, exogenous hormones or any form of treatment for prevention of miscarriage. Studies in languages other than English were also excluded where no translated version of the manuscript was available.

Threatened miscarriage was defined as patients presenting with bleeding with or without lower abdominal pain, closed internal os on cervical examination and subsequent USS confirming a viable intrauterine pregnancy (Saraswat *et al.*, 2010; NICE guidance CG154, 2012). Based on this definition of threatened miscarriage, studies that included women with pregnancy viability confirmed on an initial USS were selected for the systematic review. The primary outcome of interest was prediction of miscarriage.

Information sources and search strategy

The electronic database search included Medline (1946 to June 2015), Embase (1980 to June 2015), Cochrane library, ClinicalTrials.gov, World Health Organization international clinical trials registry, LILAC database and OpenGrey (System for Information on Grey Literature from Europe). The following MESH terms were used to create two subsets of citations (1) miscarriage (abortion, pregnancy loss, early pregnancy outcome) (2) biochemical markers (biomarkers, biological markers, hormonal markers, progesterone, β hCG, hCG, human chorionic gonadotrophin, progesterone, follistatin, CA 125, PAPP-A, activin, activin- A, inhibin, inhibin-A, estradiol, estriol, hydroxy progesterone, human placental lactogen, HPL, alpha feto protein (AFP), schwangerschafts protein (SP1), pregnancy specific beta 1 glyco protein, pregnancy zone protein (PZP)). The two subsets were

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combined using the Boolean term 'AND' to obtain a subset of citations relevant to our research question. Two authors (R.N.P. and N.P.) performed independent literature searches and the reference lists of all recent reviews and primary articles were examined to identify any articles not captured by the search. Any disagreements in selecting the papers and data extraction were resolved by consensus.

Data extraction and quality assessment

Using predetermined forms, data were extracted independently by two authors (R.N.P. and N.P.). Data were collected on study design and conduct, country of study, sample size, gestational age, biochemical markers and miscarriage prediction. From each study, outcome data were extracted in 2 × 2 tables or using the mean and SD.

Study quality assessment was performed using QUADAS-2 (Quality Assessment for Diagnostic Accuracy Studies-2: A Revised Tool) for evaluating the diagnostic accuracy of studies (Whiting *et al.*, 2011). The tool consists of four key domains covering patient selection, index test(s), reference standard, the flow and timing. Each domain was assessed in terms of risk of bias, and the first three domains were also assessed for concerns regarding applicability. Signaling questions were included in the tool to help judge the risk of bias. The index test(s) for the included studies were the biomarkers and the reference standard was miscarriage confirmed clinically or by USS or by histopathological examination during follow-up.

Statistical analysis

Statistical analysis was performed using the Cochrane systematic review software (Review Manager 5.3) and the meta-analysis of the eligible studies performed using the diagnostic test accuracy review stream (Cochrane Collaboration, 2011). Data from each primary study were summarized in a 2 × 2 table of test results and forest plots constructed showing within-study estimates and confidence interval for sensitivity and specificity of each biomarker. For biomarkers with data from four or more studies, further statistically rigorous modeling was performed using a hierarchical summary receiver operating characteristic (HSROC) model and graphs were plotted (Rutter and Gatsonis, 2001; Harbord *et al.*, 2007). The graph demonstrated summary receiver operating characteristic (SROC) curve and the prediction region, the summary point and the confidence region. The between-study heterogeneity was accounted for in the HSROC model. Posterior predictions (empirical Bayes estimates) of the sensitivity and specificity in each study were obtained and plotted since the empirical Bayes estimates give the best estimate of the true sensitivity and specificity in each study. In addition, sensitivity, specificity, positive and negative likelihood ratio for each biomarker were tabulated.

Results

Study selection

The electronic searches identified 6727 articles and further 93 articles were found from other sources and review of reference lists of individual manuscripts. After reviewing the titles and removing the duplicates 154 manuscripts were identified, of which 119 were excluded after reading the abstract. Full manuscripts of 35 articles were reviewed in detail and of these 16 studies were excluded (patient population was different in 11 studies; four studies were excluded for retrospective study design and one due to data duplication). A total of 19 studies were included in the qualitative data synthesis. Four studies (Azogui *et al.*, 1996; Vavilis *et al.*, 2001; Johns *et al.*, 2007; Jauniaux *et al.*, 2015) were further excluded in the quantitative meta-analysis as the data could not be obtained for the 2 × 2 tables. Overall, 15 studies were eligible for the quantitative meta-analysis and included 1263 women (Fig. 1). 'Of the included studies (Table I), only one study had results from use of a combination of markers (Scarpellini *et al.*, 1995); all other studies which used combination markers, could not be included in the review because they did not meet the predefined inclusion criteria (Kunz and Keller, 1976; Hertz and Schultz-Larsen, 1983; Osmanagaoglu *et al.*, 2010) (Table II).

Study characteristics

All included studies were prospective cohort studies on women with threatened miscarriage. Of the 15 studies, all excepting four (Jouppilla *et al.*, 1980; Westergaard *et al.*, 1985; Ruge *et al.*, 1990; Hanita *et al.*, 2012) included women of gestational age less than 14 weeks. The characteristics of the included studies are summarized in Table I and excluded studies in Table II.

Risk of bias assessment

The risk of bias was assessed in four main domains using the 'QUADAS-2: A Revised Tool' for patient selection, index test, reference standard and flow and timing (Fig. 2). Four of the included studies did not specify their exclusion criteria and therefore scored 'high risk' for patient selection. For the index test most studies had not specified a cut off level to differentiate between ongoing pregnancies and miscarriage, and those that had specified a cut off level had not specified it prior to starting the project. This is an area of major bias for the included studies. The reference standard for the review is occurrence of miscarriage which can be best diagnosed using USS or clinical history followed by histopathological examination of the products of conception. Some studies used telephone interviews or review of case notes to determine the outcome, which can contribute to bias. It was not clearly stated in the studies whether the reference standard was interpreted without the knowledge of the index test. However, this is unlikely to affect applicability of the studies since miscarriage is an objective diagnosis and is not prone to subjective interpretation. In the flow and timing section of the QUADAS-2 tool, although it was difficult to predict a specific time interval from the index test to reference standard (occurrence of miscarriage), we used the sampling question to see whether the patients were followed up until at least 23 weeks, so as not to miss any miscarriages (World Health Organization [WHO, 2001] has defined miscarriage as premature loss of a fetus up to 23 weeks of pregnancy and weighing up to 500 g). Therefore, quality concerns exist for the diagnostic accuracy studies included for the prediction of the miscarriage.

Quantitative data summary and synthesis of results

Data were summarized for the biomarkers serum hCG, progesterone, estradiol, PAPP-A and CA 125. Test results were tabulated in a 2 × 2 table and forest plots constructed for the sensitivity and specificity of the biomarker with their confidence intervals (CI). There were other serum biomarkers for which only single studies were available and therefore meta-analysis could not be performed. These were HPL, AFP, SP1 and PZP (Westergaard *et al.*, 1985); plasma renin activity, plasma renin substrate and sex-hormone binding globulin (Siimes *et al.*, 1983); inhibin A, activin A, follistatin (Johns *et al.*, 2007; Phupong and Hanprasertpong, 2011) and estriol (Dessaive *et al.*, 1982).

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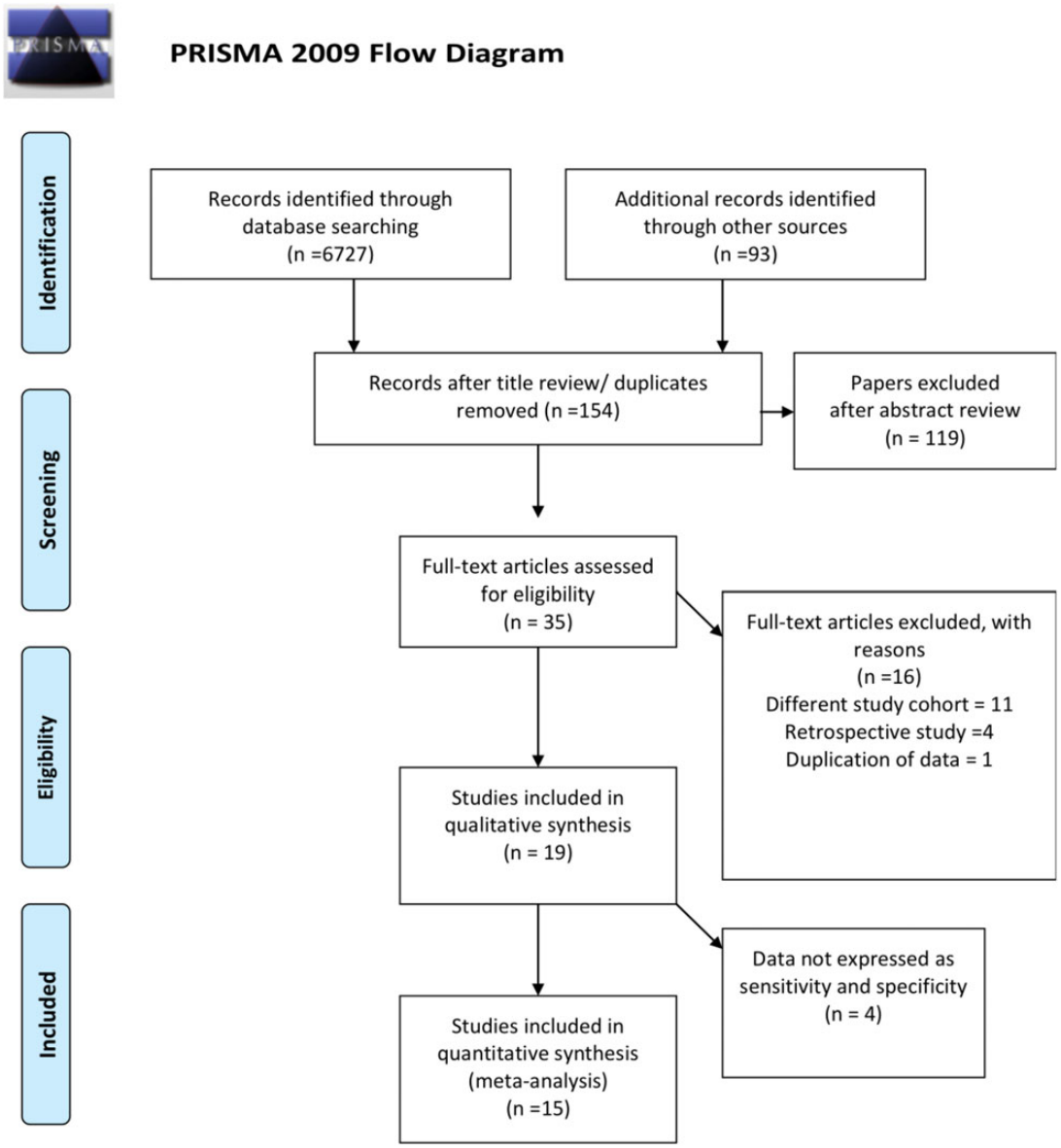

Figure 1 Flow chart for identification and selection of studies in the systematic review and meta-analysis (Moher *et al.*, 2009).

Serum hCG

There were eight studies with a total of 584 women that investigated either intact hCG (International Federation of Clinical Chemistry denotes intact hCG as 'HCG') (Stenman *et al.*, 2006) or βhCG to predict the outcome in women with threatened miscarriage. Of these, three studies used intact hCG (Stoppelli *et al.*, 1981; Siimes *et al.*, 1983; Westergaard *et al.*, 1985) and five used β hCG (Jouppilla *et al.*, 1980; Dessaive *et al.*, 1982; Scarpellini *et al.*, 1995; Leylek *et al.*, 1997; Maged and Mostafa, 2013). The forest plots were plotted separately for studies that used β hCG and intact hCG (Fig. 3a and b). Further analysis using HSROC (β hCG and intact hCG) showed a sensitivity of 44% (95% CI 17–75%), a specificity of 86% (95% CI 80–91%), a positive likelihood ratio of 3.37 (95% CI 1.98–5.74%) and a negative likelihood ratio of 0.63 (95% CI 0.36–1.11) (Table III and Fig. 3c).

Serum progesterone

Six studies with 481 women used serum progesterone to predict outcome in threatened miscarriage (Jouppilla *et al.*, 1980; Stoppelli *et al.*, 1981; Dessaive *et al.*, 1982; Westergaard *et al.*, 1985; Leylek *et al.*, 1997; Maged and Mostafa, 2013) (Supplementary Fig. S1a). Further analysis using HSROC showed a sensitivity of 30% (95% CI 2–87%), a specificity of 86% (95% CI 78–91%), a positive likelihood ratio of 2.24 (95% CI 0.32–15.80%) and a negative likelihood ratio of 0.81 (95% CI 0.35–1.86) (Table III and Supplementary Fig. S1b).

Serum estradiol

Four studies, with 244 women investigated serum estradiol to predict outcome in women with threatened miscarriage (Stoppelli *et al.*, 1981; Dessaive *et al.*, 1982; Siimes *et al.*, 1983; Westergaard *et al.*, 1985)

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Table I Characteristics of the 15 studies included in a systematic review and diagnostic accuracy meta-analysis of serum biomarkers used in the prediction of outcome in women with threatened miscarriage.

Authors and publication year	Country	Patient characteristics	Index tests (biomarkers)	Index test cutoff	Miscarriage diagnosis	Follow-up duration
Jouppilla et al. (1980)	Finland	$N = 103$, 6–20 weeks, excluded cervical causes of bleeding	β hCG, progesterone and estradiol	Not pre specified	USS	Not specified
Stoppelli et al. (1981)	Italy	$N = 62$, 5–13 weeks, excluded uterine malformations and systemic diseases	hCG, progesterone, estradiol	Not pre specified	Not specified	Not specified
Dessaive et al. (1982)	Belgium	$N = 49$, 4–12 weeks, excluded missed and incomplete miscarriage, ectopic and molar	β hCG, progesterone, estradiol and estriol	Not pre specified	USS/histology/ clinical history	Not specified
Siimes et al. (1983)	Finland	$N = 74$, <14 weeks, no exclusion criteria mentioned	hCG, estradiol, plasma renin substrate, sex-hormone binding globulin, plasma renin activity	Not pre specified	Hospital record /histology	Not specified
Westergaard et al. (1985)	Denmark	$N = 77$, 7–20 weeks, excluded blighted ovum, missed abortion, molar and ectopic pregnancy	hCG, progesterone, estradiol, PAPP-A, AFP, HPL, schwangerschafts protein 1 (SP1), pregnancy zone protein (PZP)	Not pre specified	USS	Not specified
Ruge et al. (1990)	Denmark	$N = 128$, 6–19 weeks, no exclusion criteria mentioned	PAPP-A	Not pre specified	USS	End of pregnancy
Öçer et al. (1992)	Turkey	$N = 25$, 7–12 weeks, excluded vaginitis, cervicitis, history of recurrent miscarriage and smoking	CA 125	Not pre specified	Not specified	20 weeks
Scarpellini et al. (1995)	Italy	$N = 48$, 6–11 weeks, excluded blighted ovum, ectopic, multiple pregnancy, assisted conception and those who could not be contacted or followed up	β hCG, CA 125, CA 125 + β hCG	CA 125 > 120 IU/ml	USS	24 weeks
Leylek et al. (1997)	Turkey	$N = 40$, 6–12 weeks, no exclusion criteria mentioned	β hCG, progesterone, estradiol, estriol, CA 125	β hCG 25 IU/ml, progesterone 21 ng/ml, CA125 120 IU/ml	Not specified	Not specified
Sherif et al. (2000)	Egypt	$N = 100$, 6–13 weeks. No exclusion criteria mentioned.	CA 125	>21 U/ml	USS	Not specified
Fiegler et al. (2003)	Poland	$N = 200$, 5–12 weeks, excluded ectopic pregnancy, gestational age >12 weeks, empty gestational sac, multiple pregnancy, cervical insufficiency, cervical surgery, assisted conception, history of endometriosis, ovarian abnormality and inability to detect or examine one or both ovaries by USS	β hCG, CA 125	Not pre specified	Hospital record	Until 4 weeks of discharge from hospital
Phupong and Hanprasertpong (2011)	Thailand	$N = 30$, 6–14^{+6} weeks, excluded multiple pregnancy, diabetes, hypertension, fetal and chromosomal anomaly	Inhibin A	Not pre specified	Not specified	Not specified
Hanita et al. (2012)	Malaysia	$N = 42$, 6–22 weeks, excluded missed abortion, local cause of vaginal bleeding like cervical polyp, cervical cancer or local trauma. Those with confirmed congenital anomalies, twin pregnancies and pregnant women who smoked	PAPP-A	Not pre specified	USS	Up to 22 weeks

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Table I *Continued*

Authors and publication year	Country	Patient characteristics	Index tests (biomarkers)	Index test cutoff	Miscarriage diagnosis	Follow-up duration
Maged and Mostafa (2013)	Egypt	*N* = 150, 5–12 weeks, excluded those with multiple pregnancy, missed/inevitable/incomplete miscarriage, ectopic and molar pregnancy	β hCG progesterone, CA 125	Not pre specified	Not specified	Not specified
Xie *et al.* (2014)	China	*N* = 135, first trimester, excluded multiple pregnancy, pregnancy by artificial insemination, abnormal uterine development, smoking, diabetes, hypertension	CA 125	Not pre specified	USS/telephone interview	Up to 28 weeks

PAPP-A, pregnancy associated plasma protein A; AFP, alpha feto-protein; HPL, human placental lactogen; CA 125, cancer antigen 125; USS, ultrasound scan.

(Supplementary Fig. S2a). Further analysis using HSROC showed a sensitivity of 45% (95% CI 6–90%), a specificity of 87% (95% CI 81–92%), a positive likelihood ratio of 3.72 (95% CI 1.01–13.71) and a negative likelihood ratio of 0.62 (95% CI 0.20–1.84) (Table III and Supplementary Fig. S2b).

Serum PAPP-A

Three studies with 236 women studied PAPP-A to predict miscarriage (Westergaard *et al.*, 1985; Ruge *et al.*, 1990; Hanita *et al.*, 2012). PAPP-A had a poor and wide sensitivity that ranged from 25 to 64% but a high specificity ranging from 88 to 94% (Supplementary Fig. S3).

Serum CA 125

Seven studies with 648 women investigated the accuracy of CA 125 in predicting miscarriage in women with threatened miscarriage (Öçer *et al.*, 1992; Scarpellini *et al.*, 1995; Leylek *et al.*, 1997; Sherif *et al.*, 2000; Fiegler *et al.*, 2003; Maged and Mostafa, 2013; Xie *et al.*, 2014) (Fig. 4a). Further analysis using HSROC showed a sensitivity of 90% (95% CI 83–94%), a specificity of 88% (95% CI 79–93%), a positive likelihood ratio of 7.85 (95% CI 4.23–14.60) and a negative likelihood ratio of 0.10 (95% CI 0.05–0.20) (Table III and Fig. 4b). The inverse of the negative likelihood ratio was 9.31 (95% CI 5–17.1) indicating that a negative test is likely to identify those who are likely to continue with the pregnancy. Empirical Bayes estimate gives the best estimate of the true sensitivity and specificity in each study and the estimates are shrunk toward the summary point compared with the study-specific estimates (Fig. 4b). Figure 4a shows a 0 value in the false negative group by Öçer *et al.* (1992), therefore sensitivity analysis was performed after adjusting the values for all cells and similar effect estimates were obtained. The CI for the estimates of sensitivity and specificity are not symmetric, therefore a log odds scale was used and similar results were obtained.

Further sensitivity analysis was done after excluding the study with a higher miscarriage rate (Stoppelli *et al.*, 1981), however, there were no significant differences noted in the prediction parameters for the biomarkers of hCG, serum progesterone and estradiol. The shape of the prediction region on the SROC plots indicates between-study heterogeneity, which was considerable.

Discussion

To the best of our knowledge, this is the first systematic review of various serum biochemical markers for predicting the outcome of threatened miscarriage. This review has highlighted that the biochemical markers serum progesterone, hCG, PAPP-A, estradiol and CA 125 have been studied in the prediction of outcome in women with threatened miscarriage. Interestingly, serum CA 125 is the most reliable marker for predicting the outcome of threatened miscarriage (sensitivity of 90%, specificity of 88%, positive likelihood ratio of 7.85 and negative likelihood ratio of 0.10) (Table III, Fig. 4a and b).

In this review, the positive likelihood ratio for CA 125 is closer to 10 indicating that this could be an accurate test. The only negatively reported study for CA 125 (Vavilis *et al.*, 2001) was not included in the meta-analysis as the results were presented using the statistical tool of mean and SD. Furthermore, this study included only 39 women compared with this meta-analysis, which has an aggregate sample size of 648. In view of this sample size difference it is likely that the absence of the study will not alter the results significantly. It has been shown that the chorio-decidual plate produces large amounts of CA 125 in early pregnancy and with the tropho-decidual detachment at the time of miscarriage, CA 125 is released into the bloodstream (Check *et al.*, 1990; Hornstein *et al.*, 1995; Scarpellini *et al.*, 1995). The caveat is that CA 125 is a non-specific biochemical marker of cellular activation of mesothelial derived tissues (Scarpellini *et al.*, 1995), therefore its utility as a predictor of miscarriage can become questionable. More so, in the infertility population in the presence of an endometrioma it would not be a reliable marker. In women who have had a conception following IVF, CA 125 can be raised in the presence of ovarian hyperstimulation, therefore interpretation can be difficult in these cases. In clinical practice CA 125 is often used as a tumor marker in the presence of ovarian cysts in pregnancy therefore, this should be interpreted with caution because of the additional source of CA 125 from the chorio-decidual plate.

Though several pregnancy hormones have been proposed as useful diagnostic markers for early pregnancy, hCG, the earliest detectable marker, is still the mainstay of modern pregnancy diagnosis. hCG can be detected as early as 8–11 days following ovulation (i.e. shortly after implantation) (Carmona *et al.*, 2003). The level of hCG in blood increases rapidly with a maximum level of 50 000–1 00 000 IU/ml attained at about 8–10 weeks of gestation. The consistent nature of this pattern

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Table II Characteristics of the studies excluded from the systematic review and diagnostic accuracy meta-analysis.

Author and publication year	Study design	Patient characteristics	Index test studied	Exclusion criteria
Garoff and Seppala (1975)	Prospective cohort	$N = 112$, first and second trimester, included women with PV bleed, no USS	HPL, AFP	Different study population (no USS done for fetal viability)
Kunz and Keller (1976)	Prospective cohort	$N = 65$, 6–20 weeks, excluded extra uterine and molar pregnancies and missed abortion	hCG, progesterone, estradiol, AFP and HPL	Different study population (patients treated with progesterone, benzodiazepenes and bed rest).
Jandial *et al.* (1978)	Retrospective	$N = 64$, 6–18 weeks	Pregnancy specific beta 1 glycoprotein, HPL	Retrospective study design
Duff *et al.* (1980)	Retrospective	$N = 66$, <20 weeks, women with threatened miscarriage	hCG, progesterone, estradiol, HPL, AFP, beta 1 glycoprotein and cystyl amino peptidase	Retrospective study design
Hertz and Schultz-Larsen (1983)	Prospective cohort	$N = 109$, 6–19 weeks, included pregnant women with PV bleed and on examination uterus enlarged and cervix closed, no USS	HPL, SP1 and AFP	Different study population (no USS done to check fetal viability)
Masson *et al.* (1983)	Design not clear	$N = 54$, 7–14 weeks, included symptomatic patients after clinical examination, no USS	HCG, PAPP-A, HPL, SP1	Different study population (no USS scan at recruitment to confirm viability) and study design not clear
Sugita *et al.* (1983)	Prospective cohort	$N = 214$, 4–20 weeks, included mixed population of normal, threatened and missed miscarriage	hCG, HPL and progesterone	Different study population and difficult to interpret results (results not clearly presented, levels not clearly specified)
Westergaard *et al.* (1983)	Prospective cohort	$N = 51$, 6–16 weeks, excluded pregnancies with missed miscarriage, molar, anembryonic and ectopic.	PAPP-A	Duplication of data (Same data used in Westergaard *et al.*, 1985)
Salem *et al.* (1984)	Prospective cohort	$N = 67$, 6–18 weeks, included women with PV bleed in ≤48 h, no USS	hCG, progesterone, SP1 and placental protein 5	Different study population (no USS done to check fetal viability)
Azogui *et al.* (1996)	Prospective cohort	$N = 25$, 7–12 weeks, excluded those with history of infertility/endometriosis	β hCG, estradiol, CA 125	Data could not be obtained in 2 × 2 table
La Marca *et al.* (1998)	Retrospective	$N = 45$, 6–10 weeks, excluded women with missed miscarriage, anembryonic pregnancy, history of miscarriage, thyroid disorder and infertility	hCG, TSH, Free T3, FreeT4, Immunoglobulin G, Immunoglobulin M, neutrophil and lymphocyte	Retrospective study design
Schmidt *et al.* (2001)	Prospective cohort	$N = 236$, 6–12 weeks, excluded women with acute/chronic infection, impaired hepatic/renal or other organ dysfunction, trophoblastic disease or neoplasia. Study wing 2: threatened miscarriage patients treated with oral magnesium and IM injection of estradiol caproate and progesterone Study wing 1: mixed population of patients with missed miscarriage, incomplete miscarriage, threatened miscarriage and ectopic pregnancy.	CA 125 and beta hCG	Different study population
Vavilis *et al.* (2001)	Prospective cohort	$N = 39$, 7–11 weeks, no exclusion criteria mentioned.	CA 125	Data could not be obtained in 2 × 2 table
Johns *et al.* (2007)	Prospective cohort	$N = 122$, <14 weeks, excluded multiple gestations, congenital anomalies and presence of large fibroid distorting the cavity	β hCG, progesterone, estradiol, PAPP- A, inhibin A, activin A, follistatin	Data could not be obtained in 2 × 2 table
Osmanagaoglu *et al.* (2010)	Prospective cohort	$N = 140$, 5–13 weeks, excluded multiple pregnancies, ectopic, missed miscarriage, blighted ovum, threatened miscarriage, pregnant women with prior treatment with progesterone or smokers or with diabetes mellitus, renal, trophoblastic or thrombophilic disease	β hCG, progesterone and CA 125	Different study population (threatened miscarriage population was excluded from the study)
Muttukrishna *et al.* (2011)	Retrospective	$N = 40$, first trimester	Soluble vascular endothelial growth factor receptor 1, soluble endoglin, placental growth factor	Retrospective study design

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Table II *Continued*

Author and publication year	Study design	Patient characteristics	Index test studied	Exclusion criteria
Taylor *et al.* (2011)	Prospective cohort	*N* = 45, 6–12 weeks, included asymptomatic women, no USS	β hCG, progesterone, PAPP-A and AEA (endocannabinoid anandamide)	Different study population (asymptomatic women with no USS were recruited)
Tong *et al.* (2012)	Prospective cohort	*N* = 782, 6–10 weeks, included asymptomatic women, USS FH+	β hCG, PAPP-A, anandamide and macrophage inhibitory cytokine 1	Different study population (asymptomatic women were included in the study)
Tu'uhevaha *et al.* (2013)	Retrospective	*N* = 181, 6–12 weeks, included asymptomatic women	Soluble FMS-like tyrosine kinase-1, placental growth factor and soluble endoglin	Different study population (asymptomatic women were included) and retrospective study design.
Jauniaux *et al.* (2015)	Prospective cohort	*N* = 71, 6–8 weeks, excluded multiple pregnancies, extra uterine pregnancies, hydatidiform mole, recurrent miscarriage, infertility treatment or endocrinological disorders	hCG, progesterone, PAPP-A, HSCRP	Data could not be obtained in 2 × 2 table

TSH, Thyroid stimulating hormone; T3, tri-iodo-thyronine; T4, thyroxine.

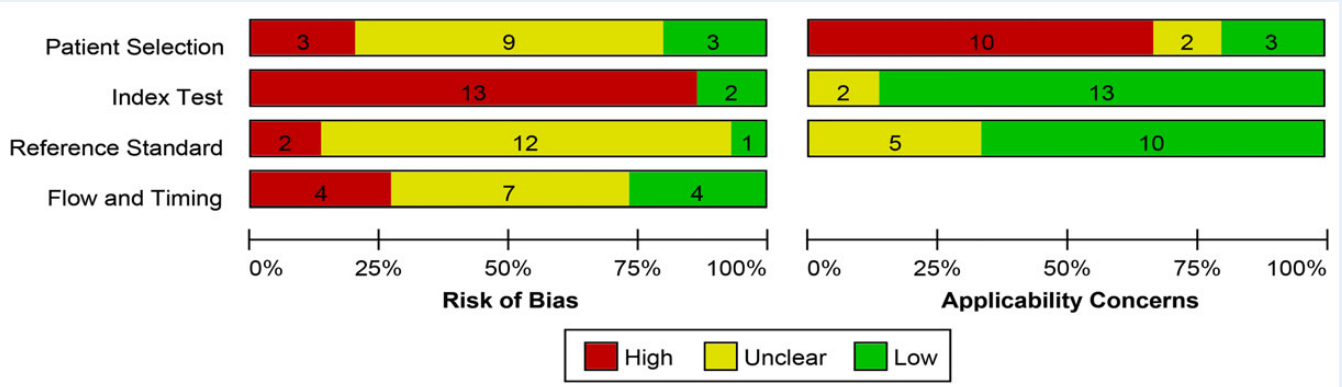

Figure 2 Summary of quality assessment of the included studies for meta-analysis using the QUADAS-2: A Revised Tool.

has made quantitative determinations of hCG a valuable tool in the clinical assessment of early pregnancy abnormalities (Duan *et al.*, 2011). hCG is a glycoprotein with a non-specific α subunit, which is similar to LH and FSH, and a β subunit which is unique to it (Stenman *et al.*, 2006). Hence some studies have used the β hCG subunit for early pregnancy prognosis (Jouppilla *et al.*, 1980; Dessaive *et al.*, 1982; Scarpellini *et al.*, 1995; Leylek *et al.*, 1997; Maged and Mostafa, 2013) and others have used intact hCG for early pregnancy prognosis (Stoppelli *et al.*, 1981; Siimes *et al.*, 1983; Westergaard *et al.*, 1985). However, it is proven that the measurement of free β hCG subunit offers no clinical advantage over measurement of intact hCG during the first half of pregnancy (Thomas *et al.*, 1990). In this meta-analysis, therefore, studies on βhCG and intact hCG were combined to create a single SROC curve (Fig. 3c).

Lin and Liu (1995) found that the sensitivity of estradiol and hCG in predicting pregnancy outcome at Week 8 of gestation was better than that of serum progesterone (80 and 85%, respectively versus 56%). We found similar results (Supplementary Fig. S1a and Fig. S2a and 3) but there was significant heterogeneity among the reported studies with regards to the sensitivity of estradiol (Dessaive *et al.*, 1982; Westergaard *et al.*, 1985).

Serum progesterone plays a crucial role in the maintenance of pregnancy via the inhibition of oxytocin-induced myometrial activity and prostaglandin excitation. Johansson (1969) was the first to demonstrate that abnormal early gestations had lower progesterone concentrations than those of viable intrauterine pregnancies. Despite these observations, because of the large biological variability of serum progesterone in early pregnancy, choosing a discriminatory value to predict viable and non-viable pregnancy is difficult (Williams *et al.*, 1992). In a systematic review conducted on a PUL population, Verhaegen *et al.* (2012) determined a serum progesterone cutoff value of 3.2–6 ng/ml to differentiate between viable and non-viable pregnancies. Most of the studies included in their review had not specified a cutoff level for serum progesterone except that of Leylek *et al.* (1997). In our review also, significant heterogeneity was noted among studies using serum progesterone to predict miscarriage. We noted that older studies (Jouppilla *et al.*, 1980;

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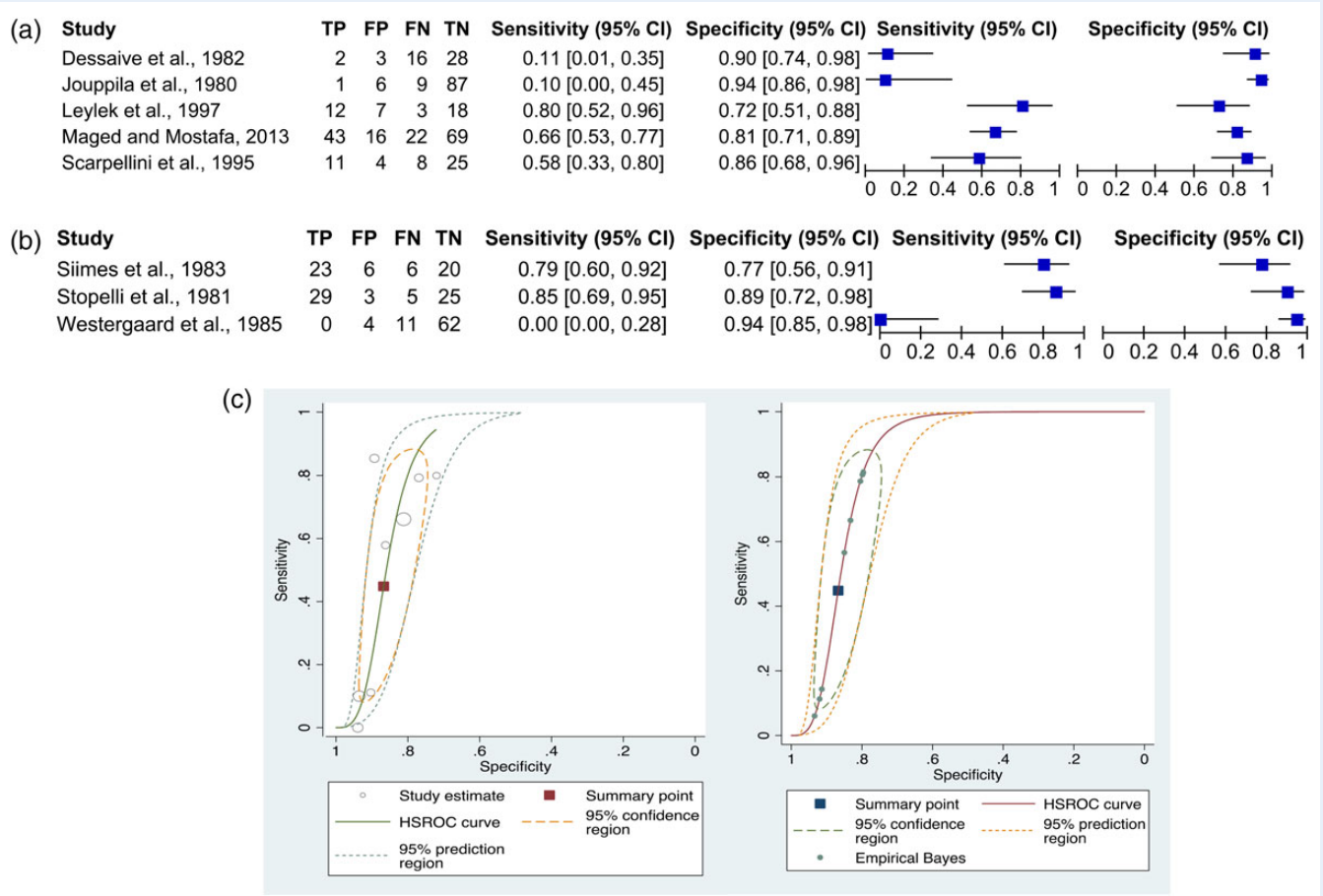

Figure 3 (**a**) Forest plot of study results for serum βhCG in women with threatened miscarriage. FN, false negative; FP, false positive; TN, true negative; TP, true positive. (**b**) Forest plot of study results for serum intact hCG in women with threatened miscarriage. FN, false negative; FP, false positive; TN, true negative; TP, true positive. (**c**) Summary receiver operating curve for hCG (intact and βhCG) and empirical Bayes estimate.

Table III Summary statistics of biochemical markers for prediction of threatened miscarriage.

Biomarker	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Serum hCG	44% (17–75)	86% (80–91)	3.37 (1.98–5.74)	0.63 (0.36–1.11)
Serum progesterone	30% (2–87)	86% (78–91)	2.24 (0.32–15.80)	0.81 (0.35–1.86)
Serum estradiol	45% (6–90)	87% (81–92)	3.72 (1.01–13.71)	0.62 (0.20–1.84)
Serum CA 125	91% (83–94)	90% (79–93)	7.86 (4.23–14.60)	0.10 (0.05–0.20)

CI, confidence interval.

Dessaive *et al.*, 1982) generally had lower sensitivity levels compared with recent studies (Leylek *et al.*, 1997; Maged and Mostafa, 2013). Overall, the results of this meta-analysis illustrate that once fetal cardiac activity is demonstrated, serum progesterone and hCG have lower diagnostic accuracy compared with other markers.

Westergaard *et al.* (1983) were the first to evaluate PAPP-A in the prediction of pregnancy outcome in women presenting with a threatened miscarriage. They concluded that PAPP-A measurement might be useful in differentiating pregnancies that will have normal outcome from those which will not (Westergaard *et al.*, 1983). The abnormal levels were frequently observed weeks before the clinical progression of spontaneous miscarriage while the fetus was still alive (Westergaard *et al.*, 1983). Ruge *et al.* (1990) observed that serum levels of PAPP-A were significantly lower in women with vaginal bleeding in early pregnancy than normal pregnant women, however they failed to differentiate between those who either later miscarried or continued with their pregnancy. PAPP-A as a biochemical marker for the prediction of early pregnancy outcome has certain limitations, which include: inability to

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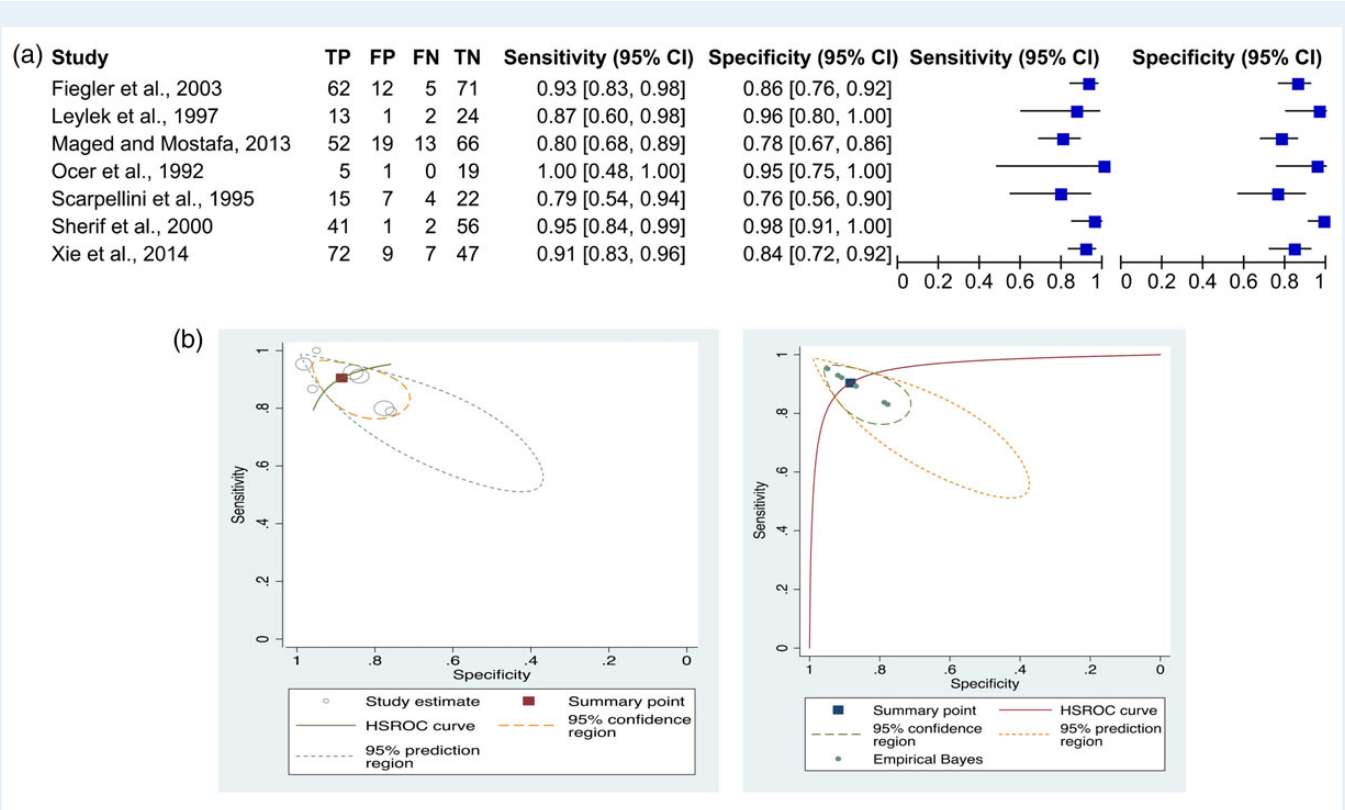

Figure 4 (**a**) Forest plot of study results for serum CA 125 in women with threatened miscarriage. FN, false negative; FP, false positive; TN, true negative; TP, true positive. (**b**) Summary receiver operating curve and empirical Bayes estimate for serum CA 125.

differentiate between normal and abnormal pregnancies at very early gestation (<6–7 weeks) (Yovich *et al.*, 1986; Ruge *et al.*, 1990); ethnic variation in serum concentrations (Spencer *et al.*, 2000; Leung *et al.*, 2006).

There is an extensive list of biomarkers that have been investigated for the prediction of early pregnancy outcome but these were not included in this meta-analysis as the studies did not meet the eligibility criteria. Some of these are activin A (Muttukrishna *et al.*, 2002; Florio *et al.*, 2007; Kirk *et al.*, 2009; Warrick *et al.*, 2012), maternal serum angiogenic factors such as placental growth factor, vascular endothelial growth factor and soluble endoglin (Ugurlu *et al.*, 2009; Muttukrishna *et al.*, 2011; Senapati and Barnhart, 2013), macrophage inhibitory growth factor (Tong *et al.*, 2012), endocannabinoids (Habayeb *et al.*, 2008; Taylor *et al.*, 2011), cytokines and chemokines (Hannan *et al.*, 2014). Johns *et al.* (2007) studied inhibin A, activin A, hCG, PAPP-A and follistatin in a threatened miscarriage population. They showed significantly lower concentrations of inhibin A, PAPP-A and hCG in those who had first trimester miscarriage compared with those who had term pregnancies. We could not include this study in our meta-analysis as the results were expressed as mean and SD. Furthermore, although a combination of biomarkers may give higher predictive value, there was only one study (Scarpellini *et al.*, 1995) that used both serum CA 125 and hCG, with a sensitivity of 78.9% and specificity of 96.5%. All other studies that used combination markers did not meet the inclusion criteria for the review (Kunz and Keller, 1976; Hertz and Schultz-Larsen, 1983; Osmanagaoglu *et al.*, 2010) (Table II).

We used demonstrable fetal heartbeat on USS as a strict inclusion criterion for the studies because prediction of miscarriage will benefit this population the most. Similarly, PUL population was excluded, as undiagnosed ectopic pregnancies could skew the outcomes.

There are a few limitations for this meta-analysis. Most of the included studies had not specified a cutoff value for the specific biochemical marker in the prediction of the outcome of miscarriage. Because of this drawback, we could only comment on the utility of each biochemical marker in predicting miscarriage and could not determine a useful 'cutoff level'. Also it is known that the levels of serum progesterone, hCG, CA 125, estradiol and serum PAPP-A change with each week of gestation. Most of the included studies did not take this into consideration. Ideally, the levels of these biochemical markers should be compared against gestation-specific normal values.

Another drawback is the quality and reporting of the included studies. The STARD (Standards for Reporting Diagnostic accuracy studies) checklist (Bossuyt *et al.*, 2003) for reporting of the diagnostic accuracy studies was published in 2003. Most of the studies included here were published before 2003 except for four (Phupong and Hanprasertpong, 2011; Hanita *et al.*, 2012; Maged and Mostafa, 2013; Xie *et al.*, 2014). The older studies have missing information and have an inadequate reporting format. Nevertheless, it is interesting that even recently published studies have pitfalls in their reporting format. The difference in reporting statistics prevented us from including some of these studies in the meta-analysis (Azogui *et al.*, 1996; Vavilis *et al.*, 2001; Johns *et al.*, 2007).

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In conclusion, biochemical markers can be used to predict the outcome of threatened miscarriage, particularly serum CA 125. Recently, high-sensitivity C-reactive protein has been studied in threatened miscarriage (Jauniaux *et al.*, 2015) and its role along with CA 125 needs to be further investigated in larger studies. In order to reliably interpret the biochemical markers in early pregnancy, gestational age-specific normograms are required and pre specified cutoff values would be important for the study design. Ultrasound markers may have a role in accurately predicting outcome of threatened miscarriage either alone or in combination with the biochemical markers. Moreover, oxidative stress markers in maternal serum or urine, such as the oxidatively modified DNA component 8-oxo-7,8-dihydro-2′-deoxyguanosine, may have a potential role in predicting miscarriage, but this needs further research. Overall, it is important to consider biomarkers that can reliably predict an ongoing pregnancy rather than predicting miscarriage since this would allay patient anxiety and be cost-effective. Future, large well-designed prospective cohort studies are needed with rigorous quality control and reporting methodology to accurately predict miscarriage outcome.

Supplementary data

Supplementary data are available at http://humupd.oxfordjournals.org/.

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Authors' roles

R.N.P. contributed to the concept, study design, database search, data extraction and quality analysis, statistical analysis, writing the manuscript and final approval of the manuscript. J.C.K. contributed to the concept, writing of the manuscript and final approval of the manuscript. D.G.T. contributed to the concept, reviewing of the manuscript and final approval of the manuscript and N.P. conceived the idea, study design, database search, data extraction and quality analysis, statistical analysis, writing the manuscript and final approval of the manuscript.

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Conflict of interest

None declared.

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