Practical Laboratory Medicine 42 (2024) e00428

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Registered Report Stage II

Exploring free pregnancy associated plasma protein a (fPAPP-A) as a biomarker in early pregnancy

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ARTICLE INFO

Keywords:
PAPP-A
Free PAPP-A
First trimester
Miscarriage
Live birth
Immunoassay

ABSTRACT

Objectives: In combined first trimester screening for Down syndrome, Pregnancy-Associated Plasma Protein A (PAPP-A) is pivotal. PAPP-A tests evaluate total PAPP-A, consisting of the biologically active free PAPP-A (fPAPP-A) and PAPP-A complexed with eosinophil major basic protein's proform (proMBP). While PAPP-A is well-researched, limited understanding persists regarding fPAPP-A's first trimester concentrations and diagnostic utility.

Design: and methods: PAPP-A and fPAPP-A levels were gauged in 602 serum samples at 2-week intervals (gestational weeks 4–14) from 159 women with delivery of a healthy neonate and 80 samples from 37 miscarriages. The final sample at the time of diagnosis from women who miscarried was included in analyses.

Results: During the first trimester, PAPP-A and fPAPP-A levels displayed significant and strong correlation ($r = 0.94$), with median values doubling weekly. Free PAPP-A constituted only 3.0 % of PAPP-A over gestational weeks. Low fPAPP-A linked to miscarriage ($p < 0.001$), maternal weight ($p < 0.001$), and smoking ($p = 0.02$). For miscarriage prediction fPAPP-A was equal to PAPP-A (area under the receiver operating characteristics curve 0.79 vs. 0.81, $p = 0.44$).

Conclusions: Investigating fPAPP-A presence and concentration directly in first trimester serum has not been done previously. This study report lower fPAPP-A values than anticipated from prior enzymatic studies of fPAPP-A. fPAPP-A was not superior to PAPP-A as a first trimester biomarker in this dataset.

1. Introduction

Globally, trends have been clear for years that fertility rates are at a steady decline due to various often irreversible factors [1]. Alongside this development, prenatal diagnosis and screening for unhealthy fetuses have improved immensely and remains a priority in many countries to try and support the declining fertility rates.

Pregnancy-associated plasma protein-A (PAPP-A) is widely used as one of two biochemical tests in the combined first trimester screening for Down syndrome (with free beta human chorionic gonadotropin (β−hCG)). The maternal PAPP-A plasma concentrations

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https://doi.org/10.1016/j.plabm.2024.e00428
Received 20 June 2024; Received in revised form 16 August 2024; Accepted 15 September 2024
Available online 16 September 2024

are affected by factors such as maternal weight, age, ethnicity, cigarette smoking, diabetes mellitus, in vitro fertilization and age [2–8]. PAPP-A mostly circulates as a complexed form with the proform of eosinophil major basic protein (proMBP) [9,10]. A smaller fraction of PAPP-A circulates as an unbound form, also called free PAPP-A (fPAPP-A) [11]. The enzymatic activity of PAPP-A is inhibited when proMBP binds PAPP-A leading to the irreversible formation of the covalent 2:2 PAPPA/proMBP complex [11,12]. PAPP-A is a metalloproteinase secreted during pregnancy by the syncytiotrophoblasts of the placenta and produced in various tissues [13–15]. It regulates insulin-like growth factors (IGF) I and II by cleaving IGF factor binding proteins 2, 4 and 5 (IGFBP-2, -4 and -5) that inhibit IGF activity [16–18]. IGF activity is crucial for fetal growth and development during pregnancy and lower maternal circulating levels of PAPP-A are found in pregnancies complicated by fetal growth restriction, ectopic pregnancy, preeclampsia, preterm birth fetal trisomies and miscarriage [19–22]. The current PAPP-A assays approved for Down syndrome screening (in Europe the CE-IVD approved assays) measure total PAPP-A (the sum of fPAPP-A and PAPP-A/proMBP complex).

The concentration of fPAPP-A in early pregnancy plasma has previously only studied using methods based on the IGFBP-cleaving activity [11,23]. The portion of active fPAPP-A in maternal plasma in the first trimester of pregnancy was found to be as high as 30 % compared to approximately 1 % in the third trimester [11,23]. As fPAPP-A represents the biologically active form of PAPP-A it could be a better predictor of pregnancy outcome in the first trimester than the currently used total PAPP-A tests.

Miscarriage is the most common complication in early pregnancy estimated to end one in four pregnancies [24,25]. Assuring viability for women who present with bleeding is a challenge as the prognostic value of currently used biomarkers is limited and follow-up scans are very often necessary [26]. In 2021 The Lancet highlighted the lack of evidence surrounding miscarriage and the need for global reform of its care [25,27] including optimized ways of predicting pregnancy complications.

An immunoassay that directly detects the fPAPP-A molecule has been previously described and utilized in studies concerning cardiac patients [28–30]. Using this immunoassay, our study aimed to explore the fPAPP-A levels in a cohort of women with first trimester pregnancies at risk of miscarriage and to compare efficacy as an outcome predictor with the standard PAPP-A assay.

2. Materials and methods

2.1. Study cohort

Serum samples were collected from 196 Caucasian women in the Copenhagen-based prospective early pregnancy (PEP) cohort during 2016–2017 in Denmark [31]. This cohort has been used for investigation of biomarker potential on more occasions despite the limited number of women who miscarried (N = 37) [32–34]. Participants were ≥18 years old, provided written consent, and had an ultrasound-confirmed intrauterine pregnancy with a single fetus ≤8 weeks' gestation. Exclusions from the PEP cohort included a history of recurrent pregnancy loss (>2), diagnosed uterine or tubal abnormalities, fertility treatment, or ongoing drug abuse. Each study visit consisted of vaginal ultrasonography to assess the development of the pregnancy as well as a questionnaire, urine and blood sampling. If the sonography confirmed a miscarriage according to the criteria of Barnhart et al. [35] the patient exited the study but the sampled blood at this final visit was included in the data. The final visit for ongoing pregnancies was conducted between 12 and 14 weeks' of gestation. A follow-up confirmed all ongoing pregnancies to be delivered as healthy neonates.

2.2. Immunoassay for fPAPP-A

The samples were analysed with an in-house direct sandwich immunoassay for fPAPP-A [28]. The capture antibody of the assay binds to an epitope only accessible on fPAPP-A, and not on PAPP-A/proMBP complex while the tracer antibody binds both fPAPP-A and PAPP-A/proMBP complex. The limit of detection (LoD) for this immunoassay is 0.4 mIU/L and limit for quantitation (CV 20 %) is 1.3 mIU/L. Within-laboratory CV is 10 % at 3.4 mIU/L and 5 % at 9.0 mIU/L [28].

The recombinant fPAPP-A standards that were used as calibrators with this assay had been calibrated against a pool of third trimester pregnancy serum using a PAPP-A assay that detects both free PAPP-A and PAPP-A/proMBP complex [36]. This pool, which had been stored frozen at −70 °C, had been previously calibrated against the WHO IRP 78/610 that is no longer available [10].

2.3. Immunoassay for PAPP-A

The samples were analysed with Kryptor Compact Plus PAPP-A assay (Thermo Scientific), which detects both forms of PAPP-A (PAPP-A/proMBP complex and fPAPP-A) [36,37]. This commercial assay is in routine use for Down's syndrome screening globally and calibrated against WHO IRP78/610 when this was still available.

2.4. PAPP-A ratio

The fPAPP-A to PAPP-A ratio was determined to examine to what extent PAPP-A can be used as a surrogate marker for fPAPP-A and to validate the earlier results based on fPAPP-A enzymatic activity. The PAPP-A ratio was calculated by dividing the measured fPAPP-A concentration of the sample with respective PAPP-A concentration.

2.5. Statistical analysis

Free PAPP-A concentrations below the limit of detection (LoD) were included in the data as concentration of LoD/2 (0.2 mIU/L).

Week-specific multiple of median (MoM) values were calculated by dividing single measurement results by the week-specific median value. Median values for gestational weeks were calculated from the normal pregnancy group. Because biomarkers are susceptible to change in gestational age, the sample concentrations were normalized with week-specific MoMs and expressed as MoM values which reduces the effect of time on the results [38].

Statistical analyses were performed with IBM® SPSS® Statistics (version 28, IBM). All p values were 2-tailed and p values < 0.05 were considered statistically significant. The normality of the distribution of the continuous variables was assessed by the Kolmogorov-Smirnov test. Due to non-normal distributions the Spearman rank-correlation was used to evaluate the association between continuous variables. Mann-Whitney *U* test was used to examine the differences in the non-normally distributed continuous variables between groups, whereas Student's t-test was used for normally distributed variables. The Chi-Square test was applied to explore the association between two categorical variables. Receiver operator characteristic (ROC) analysis was applied to examine how well compared PAPP-A assays were able to distinguish the miscarried pregnancies from the normal pregnancies. The optimal cut-off values for fPAPP-A, PAPP-A and PAPP-A ratio were calculated by using Youden's index. The areas under ROC curves were compared as described by DeLong and colleagues [39].

2.6. Ethical considerations

The PEP cohort study obtained consent from both the Danish Data Protection Agency (NOH-2015-042, 04306) and the local Regional Scientific Ethics Committee (H-15018030). Human participants were enrolled and treated in compliance with the principles outlined in the Declaration of Helsinki and were given the option to opt-in for the storage of their blood samples for future research. Only participants who gave permission for future research were included in this study.

3. Results

3.1. Study cohort

Baseline characteristics are described in Table 1. The cohort included 159 women with normal pregnancies (81 %) and 37 women with miscarriages (19 %). Women with miscarriages were all seen at the first visit (between 4 and 8 weeks' gestation), 36 were seen at

Table 1
Characteristics of the study cohort divided by pregnancy outcome.

	Normal pregnancy	Miscarriage	*p*
	N = 159	N = 37	
Maternal age, *years* (mean (SD))	29.3 (4.3)	31.2 (5.3)	0.05[a]
Maternal age group (*n*, %)			0.06[b]
<30	97 (61)	18 (47)	
30-35	46 (29)	10 (28)	
>35	16 (10)	9 (25)	
BMI, kg/m^2 (mean (SD))[a]	24.46 (4.3)	25.17 (4.9)	0.4[a]
BMI group (*n*, %)[a]			0.7[b]
Normal weight (BMI 18.6–25)	94 (61)	21 (58)	
Overweight (BMI >25)	61 (39)	16 (44)	
Smoking status (*n*, %)			0.7[b]
Never	90 (57)	24 (66)	
Previously	27 (17)	4 (11)	
Stopped <6 months	30 (18)	6 (17)	
Current	12 (8)	2 (6)	
Birthweight, *g* (mean (SD))	3553 (523)	NA	
Samples (*n*, %)			
All weeks	602	80	
GA 4 weeks	10 (1.7)	2 (2.5)	
GA 5 weeks	36 (6.0)	9 (11.3)	
GA 6 weeks	70 (11.6)	19 (23.8)	
GA 7 weeks	77 (12.8)	17 (21.3)	
GA 8 weeks	85 (14.1)	10 (12.5)	
GA 9 weeks	79 (13.1)	17 (21.3)	
GA 10 weeks	78 (13.0)	4 (5.0)	
GA 11 weeks	68 (11.3)	1 (1.3)	
GA 12 weeks	56 (9.3)	1 (1.3)	
GA 13 weeks	30 (5.0)	NA	
GA 14 weeks	12 (2.0)	NA	
GA 15 weeks	1 (0.2)	NA	

GA: Gestational age, NA: Not applicable.
Underweight (BMI<18.5) women, n = 5 were excluded.
[a] Student's t-test.
[b] Chi-square test.

the second (between 6 and 9 weeks' gestation), 13 at the third (between 8 and 11 weeks' gestation) and 2 at the fourth visit (between 11 and 13 weeks' gestation). Among the miscarriage group, 90 % of samples were taken during gestational weeks 5–9. Women who miscarried were slightly older ($p = 0.05$), while no significant differences were found in BMI or smoking status ($p = 0.52$ and $p = 0.65$, respectively). A total of 682 serum samples were analysed: 602 from normal pregnancies and 80 from miscarriages. For normal pregnancies, samples were collected between weeks 4–15, with 58 % from weeks 5–9.

3.2. fPAPP-A serum levels in the first trimester of normal pregnancy

The fPAPP-A serum levels increased throughout the first trimester in normal pregnancies (Fig. 1).

During the gestational weeks 4, 5 and 6, most women had serum fPAPP-A concentrations below the limit of detection (LoD) (83 %, 91 % and 64 % of measurements, respectively). Thereafter the median serum level of fPAPP-A approximately doubled every week. The median serum level of fPAPP-A at week 7 was 2.4 mIU/L and at week 14 it was 214.9 mIU/L. There was negligible correlation between fPAPP-A and birthweight ($r = -0.03$, $p = 0.47$).

3.3. Impact of maternal parameters on fPAPP-A serum levels

3.3.1. Age

There was negligible negative correlation between fPAPP-A (MoM) and maternal age ($r = -0.05$, $p = 0.19$). Similarly, there was no difference in the median fPAPP-A serum levels between maternal age categories (Fig. 2), neither in normal pregnancies (median varied between 1.0 and 1.1 MoM) nor in miscarriages (median varied between 0.5 and 1.0 MoM).

3.3.2. Body mass index (BMI)

Women were divided into normal weight (excluding BMI <18.5 kg/m^2) and overweight (BMI above 25 kg/m^2). The underweight women were excluded from the analyses due to low number of observations. A weak negative correlation between BMI and fPAPP-A ($r = -0.31$, $p < 0.001$) was observed. As seen in Fig. 2, this difference was also clear when stratifying the results by BMI groups normal and overweight and reached statistical significance ($p = 0.002$ and $p = 0.023$, respectively). The median fPAPP-A (MoM) among normal weight women were 1.1 MoM (normal pregnancy) and 1.0 MoM (miscarriage) compared to the lower fPAPP-A (MoM) values among overweight women, 0.9 MoM (normal pregnancy) and 0.3 MoM (miscarriage).

3.3.3. Smoking

Women were divided into two groups; non-smokers (never and previously) and smokers (stopped <6 months and current).

In normal pregnancies there was no difference in fPAPP-A (MoM) between the non-smokers and smokers (median MoM 1.0 vs. 1.0, $p = 0.85$) (Fig. 2).

However, in miscarried pregnancies the fPAPP-A was markedly lower in smokers (median MoM 1.0 vs. 0.1, $p = 0.02$).

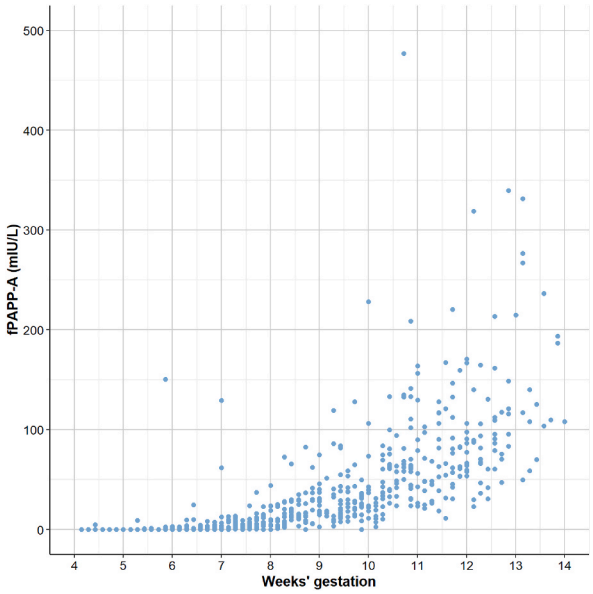

Fig. 1. Scatter plot of all 602 measured fPAPP-A serum concentrations against gestational age for normal pregnancies in the first trimester.

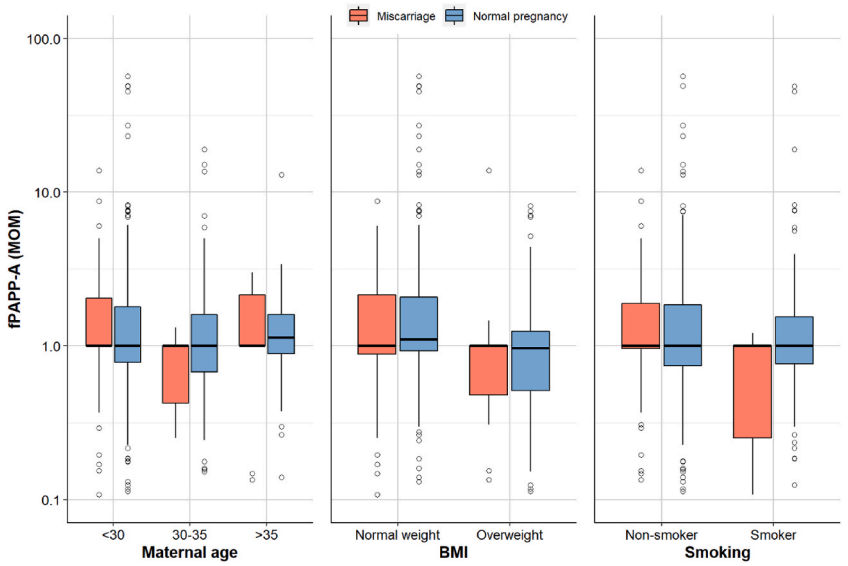

Fig. 2. Distribution of fPAPP-A (MoM) by maternal age, BMI and smoking status categories, separately for miscarried pregnancies (red) and for normal pregnancies (blue) (samples of all weeks). Whiskers represent the length of the lower and upper tails of fPAPP-A distribution; boxes represent 25th percentile, median, and 75th percentile; open circles represent outliers.

3.4. fPAPP-A:PAPP-A ratio

On average 2–3 % of PAPP-A was in free form throughout the first trimester of pregnancy (Fig. 3).

There was a trend of increasing PAPP-A ratio in time during all gestational weeks both for normal pregnancies and miscarriages ($r = 0.29$, $p < 0.001$ and $r = 0.10$, $p = 0.39$, respectively), especially for gestational weeks 7–10 ($r = 0.21$, $p < 0.001$ and $r = 0.37$, $p = 0.01$, respectively).

There was no significant difference in PAPP-A ratio (MoM) values when all weeks were included in the analysis ($p = 0.72$), but in weeks 7–10 normal pregnancies showed a higher ratio than miscarried (median 1.0 vs. 0.8 MoM, respectively, $p < 0.002$).

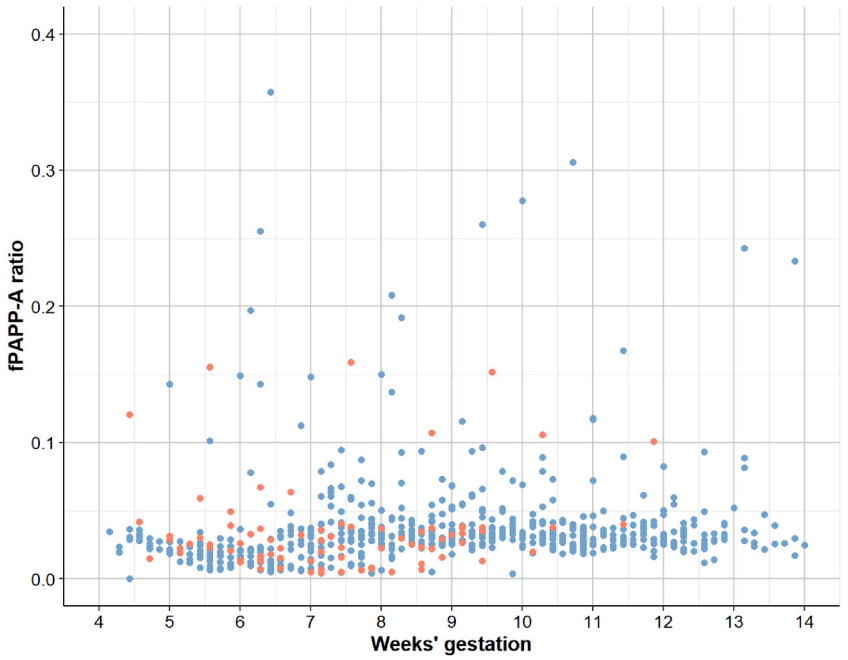

Fig. 3. Scatter plot of PAPP-A ratio (fPAPP-A/PAPP-A) against gestational age for normal (blue circle) and miscarried pregnancies (red circle) in the first trimester. In color.

3.5. Comparison of fPAPP-A levels in normal pregnancy and in miscarriage

To assess fPAPP-A as a predictor of miscarriage risk in the first trimester, fPAPP-A serum levels were compared between normal and miscarried pregnancies. Observations were limited to 7–10 weeks, ensuring sufficient data above the LoD in both groups.

Throughout the first trimester, fPAPP-A levels increased in both normal and miscarried pregnancies but were significantly lower in the latter (Fig. 4).

The relative difference between the two groups decreased over time. At week 7, the median fPAPP-A level in miscarried pregnancies was 92 % lower than in normal pregnancies, decreasing to 86 % at week 8, 68 % at week 9, and 43 % at week 10. Conversely, PAPP-A levels did not exhibit a similar temporal trend (63 %, 78 %, 66 %, and 74 % at gestational weeks 7–10, respectively). Mann-Whitney *U* test confirmed a statistically significant difference in fPAPP-A levels between the two groups at each studied gestational week ($p < 0.001$).

3.6. fPAPP-A and PAPP-A as predictors of miscarriage in pregnancy

Free PAPP-A and PAPP-A were compared as predictors of miscarriage in the first trimester. ROC analysis assessed their ability to differentiate miscarried pregnancies from normal pregnancies.

Fig. 5 shows a strong correlation between fPAPP-A and PAPP-A in both normal pregnancy and miscarriages ($r = 0.95$ and $r = 0.83$, respectively). PAPP-A increased similarly to fPAPP-A throughout the first trimester. Both fPAPP-A (MoM) and PAPP-A (MoM) weakly correlated with maternal age, BMI, and birthweight ($r = 0.03$, $r = -0.30$, and $r = -0.08$, respectively).

Both fPAPP-A (MoM) and PAPP-A (MoM) effectively distinguished miscarried pregnancies. ROC curve analysis from weeks 4–15, 7–10, and 7–8 showed the highest AUC values for both markers at 7–8 weeks. The AUC for fPAPP-A was 0.79 and for PAPP-A was 0.81. The difference in area under the ROC curves between fPAPP-A and PAPP-A was not statistically significant ($p = 0.44$). The optimal cut-off value for fPAPP-A was 0.39 MoM and for PAPP-A was 0.50 MoM. At these thresholds, fPAPP-A showed 77 % sensitivity and 74 % specificity, while PAPP-A exhibited 80 % sensitivity and 78 % specificity.

Regarding PAPP-A ratio (MoM), the highest AUC in ROC analysis was also achieved at 7–8 weeks' gestation but performed significantly worse than fPAPP-A and PAPP-A (PAPP-A ratio AUC 0.67, $p = 0.01$ and $p = 0.05$, respectively).

4. Discussion

This is the first study to examine the fPAPP-A serum concentrations during the first trimester using a specific immunoassay. Previous studies have observed increasing PAPP-A levels throughout pregnancy, doubling every 3–4 days and declining post-delivery [40]. However, direct measurement of fPAPP-A in the first trimester has not been previously reported. Our findings demonstrate that fPAPP-A serum levels increase with gestation, doubling approximately weekly after 6 weeks.

The basic observations of fPAPP-A variations during first trimester overall parallel those seen for PAPP-A. The negative correlation between fPAPP-A and maternal age and BMI in normal pregnancies was weak which is equivalent to what has been seen for PAPP-A [4,

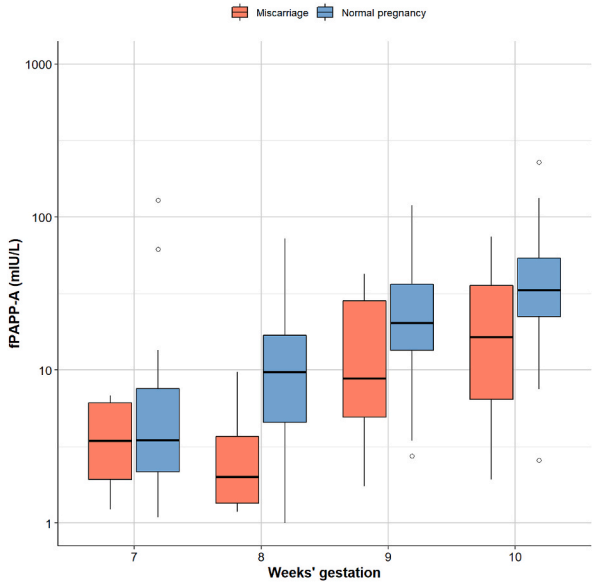

Fig. 4. Distribution of fPAPP-A concentration by gestational week, separately for miscarried pregnancies (red) and normal pregnancies (blue). Whiskers represent the length of the lower and upper tails of fPAPP-A distribution; boxes represent 25th percentile, median, and 75th percentile; open circles represent outliers.

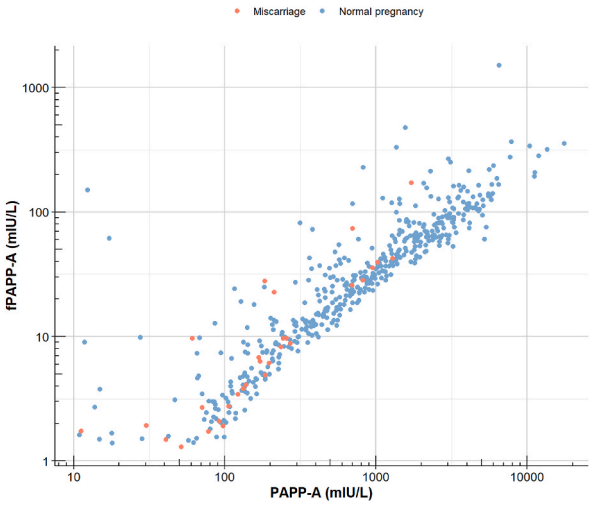

Fig. 5. Logarithmic scatter plot of fPAPP-A concentration against PAPP-A concentration separating miscarriages (red) and normal pregnancies (blue). Samples of all weeks only showing values above the fPAPP-A limit of quantification (1,3 mIU/L, CV% <20 %).

41]. Free PAPP-A levels were decreased in women who smoked compared to non-smokers, and women who miscarried measured even lower values. Smoking is linked to impaired placentation and increased miscarriage risk [42–44]. The expected association was not found in this study, most likely representing a type II statistical error due to the limited number of miscarriages – and smokers.

Lower levels of PAPP-A are associated with pregnancy complications and reduced birthweight [45–47]. However, in this study, fPAPP-A showed only negligible negative correlation to birthweight, again possibly due to the limited sample size.

In miscarried pregnancies, the fPAPP-A levels were significantly lower than in normal pregnancies, suggesting potential issues in pregnancy progression and an early indicator of impending miscarriage. Reduced PAPP-A levels are linked to aneuploidy, the main cause of miscarriage [48]. However, measuring fPAPP-A levels during 4–6 weeks' gestation, when miscarriage risk is highest, proved challenging as most concentrations fell below the assay's limit of detection. In contrast, PAPP-A levels remained measurable even in early gestational weeks.

In 2007, Gyrup and colleagues [23] using an enzymatic activity assay estimated up to 30 % of PAPP-A could be in free form in the first trimester, but only about 1 % in the third. The approach included separating PAPP-A from samples with a capture antibody attached to a microtitration well and exposing to the captured PAPP-A an IGFBP-4 derived peptide labelled with a fluorophore at one end and a quencher at the other end. The rate of increasing fluorescence signal indicated the presence and amount of active PAPP-A in the sample. Conversely, in our study the fraction of fPAPP-A in the first trimester was only 2–3% of PAPP-A (median) in both normal and miscarried pregnancies. We also found little variation in the fPAPP-A to PAPP-A ratio during the first trimester which is consistent with the almost parallel increase in both proMBP and PAPP-A found in previous publications by Christiansen [49] and Sorensen [50] (see suppl data section); even though the proMBP to PAPP-A ratio is about 4–5 times higher at GA week 7 than in GA week 13.

The differences between Gyrup's study and ours may be attributed to several factors. Gyrup measured IGFBP-4 cleaving activity of PAPP-A, while we detected the presence of fPAPP-A molecules. It has been suggested that the activity of PAPP-A detected in pregnancy serum could be due to a hypothetical partially inhibited 2:1 PAPP-A:proBMP complex [11]. The fPAPP-A epitopes may be sterically masked also in this complex rendering fPAPP-A undetectable by the fPAPP-A immunoassay. Our method and the method by Gyrup utilized differently produced recombinant PAPP-A preparations for calibration, and fPAPP-A concentrations of these recombinant PAPP-A preparations were measured with different PAPP-A immunosassays using different antibodies. In both studies the fPAPP-A concentration of recombinant PAPP-A calibrators were defined by the PAPP-A immunoassays in relation to late pregnancy serum traceable to the old WHO IRP 78/610. There can be differences in how the immunoassay epitopes are detected on differently produced recombinant proteins and endogenous proteins. Moreover, the enzymatic activity of a recombinantly produced protein may differ from the activity of endogenous protein in serum. In addition, PAPP-A may interact with other inhibitors and molecules [51–53] which may affect the detection by immunoassays and activity assays differently. Tuunainen et al. (2018) [28] showed that fPAPP-A was stable in serum after multiple freeze-thaw cycles and in various storing conditions making degradation of fPAPP-A an unlikely explanation for the low fPAPP-A/PAPP-A ratio found in this study.

Our findings align with previous knowledge about fPAPP-A, which binds to cell surfaces via glucosaminoglycan binding sites [54] This localization allows PAPP-A's enzymatic activity to be near its placental site of production during pregnancy. Circulating fPAPP-A is known to be rapidly cleared from the bloodstream [55,56], likely due to binding to vascular cell surfaces as administration of heparin induces a release of PAPP-A to the circulation, followed by rapid clearance at the rate of heparin clearance [55,56]. In contrast, the inactive PAPP-A/proMBP complex does not bind to cell surfaces [55]. The low fraction of fPAPP-A in circulation may represent spillover from the highly produced placental PAPP-A. Rapid clearance helps maintain a low circulating fPAPP-A fraction.

Regarding first-trimester outcome prediction, PAPP-A appeared slightly superior to fPAPP-A, offering higher sensitivity and specificity. PAPP-A levels were significantly higher than fPAPP-A levels early in pregnancy, facilitating the detection of abnormally

low PAPP-A levels. The difference in median fPAPP-A plasma levels between normal and miscarried pregnancies diminished in gestational weeks 7–10, potentially limiting fPAPP-A's use in predicting other pregnancy abnormalities later in the first trimester. Therefore, our results suggest that fPAPP-A is not a superior predictor of miscarriage compared to PAPP-A, and low PAPP-A levels are not specific to miscarriage but indicate the need for closer monitoring of pregnancies.

Our inability to precisely determine when the miscarriage occurred is a limitation of the cohort, as diagnosis requires ultrasound confirmation. For the calculations in this study, we opted to include the final recording of data (at the time of diagnosis) as it provides a valuable way to compare dramatic changes of the biomarker between miscarried and ongoing pregnancies. Miscarriage can have various causes, and many of the known biomarkers remain unaltered, despite fetal developmental issues.

While future research on fPAPP-A in Down's syndrome and preeclampsia screening might be interesting, our findings suggest that replacing PAPP-A with fPAPP-A in current screening algorithms would likely not significantly enhance their effectiveness. Free PAPP-A could potentially identify a small high-risk subgroup, but verifying this would require large cohort studies beyond our current scope.

5. Conclusion

In conclusion, fPAPP-A and PAPP-A could both be used to estimate the risk of miscarriage, with fPAPP-A levels lower in miscarried pregnancies. However, there was no clinically significant difference between the two biomarkers, and since PAPP-A assays are already commercially available, the use of fPAPP-A would not bring additional benefit.

Trial registration;

ClinicalTrials.gov identifier: NCT02761772

Funding information

The PEP cohort was funded by generous grants from the North Zealand Hospital Research Council, the Gangsted Foundation, the Foundation for Development of Danish Private Practice, the Tvergaard Foundation, the AP Møller Foundation, the Foundation from Danish Doctors Pension, Copenhagen University and the Danish Southern and Zealand Region. No funders were involved in the design, acquisition, analyses, or interpretation of data prior to submission.

CRediT authorship contribution statement

Jesper Friis Petersen: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Vilma Tiittanen:** Writing – review & editing, Visualization, Software, Project administration, Investigation, Formal analysis, Data curation. **Saara Wittfooth:** Writing – review & editing, Validation, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ellen Løkkegaard:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition. **Lennart Jan Friis-Hansen:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The PAPP-A antibody 3C8 used in this study was a kind gift from HyTest Ltd.

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