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First-trimester screening for trisomies in pregnancies with vanishing twin

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KEYWORDS: first-trimester screening; nuchal translucency; serum free β-hCG; serum PAPP-A; trisomy 21; vanishing twin

CONTRIBUTION

What are the novel findings of this work?
This study comparing maternal serum free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) levels at 11–13 weeks' gestation in 528 pregnancies with a vanishing twin and 5280 normal singleton pregnancies, demonstrated that, in vanishing-twin pregnancies, median free β-hCG multiples of the median (MoM) was not significantly different from that in normal singleton pregnancies, but PAPP-A MoM was higher both in the group with an empty gestational sac and in those with a dead embryo; in the latter group, the effect of a vanishing twin on PAPP-A MoM is larger the closer the demise of the twin is to blood sampling.

What are the clinical implications of this work?
First-trimester screening for trisomy in pregnancies with a vanishing twin should rely on a combination of maternal age, fetal nuchal translucency thickness and serum free β-hCG without the use of serum PAPP-A. Alternatively, PAPP-A can be included but only after appropriate adjustment for the estimated interval between gestational age at embryonic demise and blood sampling.

ABSTRACT

Objectives *To examine multiples of the median (MoM) values of serum free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in a large series of pregnancies with a vanishing twin, determine the association of these values with the interval between embryonic death and blood sampling, and develop a model that would allow incorporation of these metabolites in first-trimester combined screening for trisomy.*

Methods *This was a retrospective study comparing maternal serum free β-hCG and PAPP-A levels at 11–13 weeks' gestation in 528 dichorionic pregnancies with a vanishing twin, including 194 (36.7%) with an empty gestational sac and 334 (63.3%) with a dead embryo, with those in 5280 normal singleton pregnancies matched for method of conception and date of examination. In vanishing-twin pregnancies with a dead embryo, marker levels were examined in relation to the estimated time between embryonic death and maternal blood sampling.*

Results *First, in pregnancies with a vanishing twin, median free β-hCG MoM was not significantly different from that in normal singleton pregnancies (1.000; 95% CI, 0.985–1.016* vs *0.995; 95% CI, 0.948–1.044;* $P = 0.849$*). Second, PAPP-A MoM was higher in vanishing-twin pregnancies than in normal singleton pregnancies (1.000; 95% CI, 0.985–1.015), both in the group with an empty gestational sac (1.165; 95% CI, 1.080–1.256;* $P = 0.0001$*) and in that with a dead embryo (1.175; 95% CI, 1.105–1.249;* $P < 0.0001$*). Third, in vanishing-twin pregnancies with a dead embryo, PAPP-A MoM was related inversely to the interval between estimated gestational age at embryonic demise and blood sampling (*$P < 0.0001$*). Fourth, in first-trimester screening for trisomy 21 in singleton pregnancies, the estimated detection rate, at a 5% false-positive rate, was 82% in screening by a combination of maternal age and fetal nuchal translucency thickness, and this increased to 86% with the addition of serum free β-hCG and to 91% with the addition of serum PAPP-A. Fifth, similar performance of screening can be achieved in pregnancies with a*

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vanishing twin, provided the appropriate adjustments are made to the level of PAPP-A for the interval between estimated gestational age at embryonic demise and blood sampling.

Conclusions *First-trimester screening for trisomy in pregnancies with a vanishing twin should rely on a combination of maternal age, fetal nuchal translucency thickness and serum free β-hCG, as in singleton pregnancy, without the use of serum PAPP-A. Alternatively, PAPP-A can be included but only after appropriate adjustment for the interval between estimated gestational age at fetal demise and blood sampling.*

INTRODUCTION

In singleton pregnancies, screening for trisomy 21 by a combination of maternal age, fetal nuchal translucency thickness (NT) and serum free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11–13 weeks' gestation identifies about 90% of affected fetuses at a false-positive rate (FPR) of 5%[1]. In twin pregnancies, effective screening can also be achieved by the first-trimester combined test but the measured levels of serum free β-hCG and PAPP-A need to be adjusted for chorionicity and gestational age[2], in addition to maternal factors, as in singleton pregnancies[3]. More effective screening for trisomy 21 in singleton pregnancies is provided by analysis of cell-free DNA (cfDNA) in maternal blood, with a detection rate (DR) of > 99% and FPR of < 0.1%[4]. cfDNA testing can also be used in twin pregnancies and, although the total number of reported cases that were examined is small, the performance of screening may be similar to that reported in singleton pregnancies, but at a higher failure rate, particularly in pregnancies conceived by *in-vitro* fertilization (IVF)[5–10]. However, cfDNA testing cannot be carried out in vanishing-twin pregnancies because of flooding of cfDNA into the maternal plasma from the necrotic cytotrophoblasts, which may persist for at least 15 weeks after fetal demise[11–13].

Studies in vanishing-twin pregnancies have reported contradictory results concerning serum levels of free β-hCG and PAPP-A and the extent to which these metabolites could be included in first-trimester screening for trisomy. One study of 41 cases of a vanishing twin reported that death of one twin within 28 days prior to blood sampling was associated with increased multiples of the median (MoM) values of both free β-hCG and PAPP-A[14]. In contrast, a study of 56 cases of a vanishing twin reported that MoM values of free β-hCG and PAPP-A were not altered[15]. A third study in 270 cases of a vanishing twin reported that MoM values of free β-hCG were not altered; PAPP-A levels were not altered in vanishing twins with an empty gestational sac, but, when the second gestational sac contained a dead embryo, PAPP-A was increased and the size of the increase was related to the time interval between embryonic death and blood sampling[16]. A fourth study in 137 cases of a vanishing twin reported that MoM values of PAPP-A were increased in pregnancies with embryonic death occurring within 28 days prior to blood sampling, but not in those with demise at > 28 days prior to sampling[17].

The objectives of this study were to examine MoM values of serum free β-hCG and PAPP-A in a large series of pregnancies with a vanishing twin, determine the association of these levels with the interval between embryonic death and blood sampling, and develop a model that would allow incorporation of these metabolites in first-trimester combined screening for trisomy.

METHODS

This was a retrospective study of data collected from routine screening for trisomy by a combination of maternal age, fetal NT and serum free β-hCG and PAPP-A at 11–13 weeks' gestation, at King's College Hospital, London, UK (May 2016 to December 2018), the Fetal Medicine Centre, London, UK (August 1999 to December 2018) and Shterev Hospital, Sofia, Bulgaria (February 2010 to August 2018). We recorded maternal characteristics, including age, racial origin (white, black, South Asian, East Asian or mixed), method of conception (natural, assisted requiring the use of ovulation drugs or IVF), cigarette smoking during pregnancy and parity (parous or nulliparous if no previous pregnancy at or after 24 weeks' gestation), and measured maternal weight and height. Serum free β-hCG and PAPP-A were measured on the same day as the ultrasound examination, using a Delfia Xpress system (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA), BRAHMS KRYPTOR system (Thermo Fisher Scientific, Hennigsdorf, Germany) or Cobas e411 system (Roche Diagnostics, Penzberg, Germany). An ultrasound scan was carried out at 11 + 0 to 13 + 6 weeks to determine gestational age from the measurement of fetal crown–rump length (CRL)[18], diagnose any major fetal abnormalities and measure fetal NT. Patient characteristics, results of the investigations and pregnancy outcome were recorded in a fetal database.

The fetal database of the three participating centers were searched to identify pregnancies with a vanishing twin diagnosed at the 11–13-week scan that resulted in the live birth of a non-malformed neonate. In the vanishing-twin pregnancies, we recorded chorionicity[19] and whether the second gestational sac was empty or contained a visible dead embryo, in which case CRL was recorded. Each case of a vanishing twin was matched to 10 normal singleton pregnancies resulting in the live birth of a non-malformed neonate, for method of conception and date of examination (difference of within 15 days).

This study constitutes a retrospective analysis of data derived from a routine clinical service and did not require ethics committee approval.

Statistical analysis

Data from categorical variables are presented as *n* (%) and those from continuous variables as mean and 95% CI. The measured serum concentrations of

free β-hCG and PAPP-A were standardized into MoM values, adjusted for gestational age, maternal weight, height, racial origin, parity, smoking status and method of conception[2,3]; MoMs were further adjusted for study site and machine, over time, when appropriate. The median MoM values of free β-hCG and PAPP-A in vanishing-twin pregnancies were compared with those in normal singleton pregnancies by *t*-tests.

In vanishing-twin pregnancies with a CRL measurement of the dead embryo, the mean $\log_{10}$ MoM was assumed to depend linearly on time since demise; this linear relationship was assumed to continue until the mean $\log_{10}$ MoM equaled that in vanishing-twin pregnancies with an empty sac. Beyond this, the mean was taken to be constant at this value for all vanishing twins. On correcting MoM values for pregnancies with a vanishing twin, MoM diagnostics were assessed by diagnostic plots. The estimated gestational age at embryonic death was derived from the embryonic CRL[20].

Modeled performance of screening for trisomy 21 in singleton pregnancies by combinations of fetal NT and serum free β-hCG and PAPP-A was assessed using Monte Carlo methods to sample from the modeled Gaussian distributions[3,21]. The predictive performance of first-trimester screening for trisomy 21 was assessed using receiver-operating-characteristics (ROC)-curve analysis.

The statistical software package R was used for data analyses[22].

RESULTS

Vanishing twin at 11–13 weeks' gestation

The study population comprised 528 dichorionic pregnancies with a vanishing twin, including 194 (36.7%) with an empty gestational sac and 334 (63.3%) with a dead embryo, and 5280 normal singleton pregnancies. Maternal characteristics of the study population are summarized in Table 1. The vanishing-twin pregnancies included 140 from King's College Hospital, 291 from the Fetal Medicine Centre and 97 from Shterev Hospital.

In pregnancies with a vanishing twin, median free β-hCG MoM was not significantly different from that in normal singleton pregnancies (1.000; 95% CI, 0.985–1.016 *vs* 0.995; 95% CI, 0.948–1.044) ($P = 0.849$) (Figure 1). In contrast, PAPP-A MoM was higher in vanishing-twin pregnancies than in controls (1.000; 95% CI, 0.985–1.015), both in the group with an empty gestational sac (1.165; 95% CI, 1.080–1.256; $P = 0.0001$), and in those with a dead embryo (1.175; 95% CI, 1.105–1.249; $P < 0.0001$); in the latter group, the effect of a vanishing twin on PAPP-A MoM was larger the closer the demise of the

Table 1 Maternal and pregnancy characteristics of study population of pregnancies with vanishing twin and normal singleton pregnancies (controls)

Characteristic	*Controls (n = 5280)*	*Vanishing twin (n = 528)*
GA at blood sample (weeks)	12.4 (12.4–12.5)	12.6 (12.6–12.7)
Maternal age (years)	35.3 (35.2–35.5)	36.5 (36.1–36.9)
Maternal weight (kg)	65.7 (65.3–66.0)	68.0 (66.8–69.1)
Racial origin		
White	4568 (86.5)	455 (86.2)
Black	290 (5.5)	27 (5.1)
South Asian	261 (4.9)	29 (5.5)
East Asian	94 (1.8)	8 (1.5)
Mixed	67 (1.3)	9 (1.7)
Method of conception		
Natural	1943 (36.8)	189 (35.8)
In-vitro fertilization	3145 (59.6)	320 (60.6)
Ovulation drugs	192 (3.6)	19 (3.6)
Smoker	134 (2.5)	9 (1.7)
Parity		
Parous	2055 (38.9)	201 (38.1)
Nulliparous	3225 (61.1)	327 (61.9)
Empty gestational sac	—	194 (36.7)

Data are given as mean (95% CI) or *n* (%). GA, gestational age.

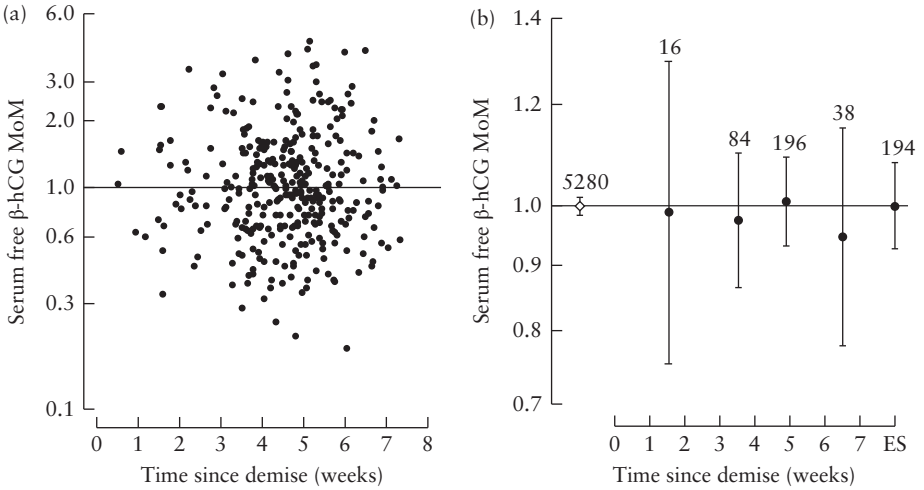

Figure 1 (a) Individual values of serum free beta-human chorionic gonadotropin (β-hCG) multiples of the median (MoM) in vanishing-twin pregnancies and (b) median values with 95% CI and *n* in vanishing-twin pregnancies (●), according to interval between embryonic demise and blood sampling, and in normal singleton pregnancies (◇). ES, empty gestational sac.

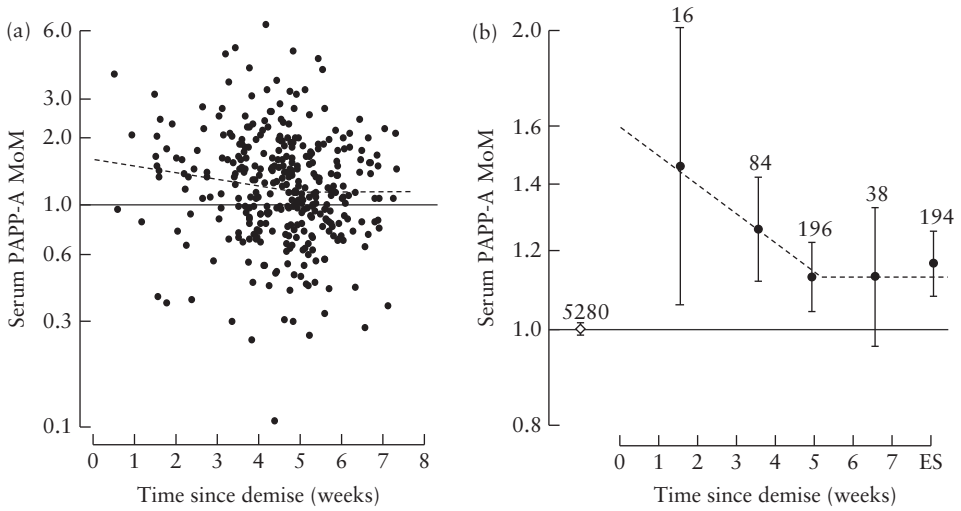

Figure 2 (a) Individual values of serum pregnancy-associated plasma protein-A (PAPP-A) multiples of the median (MoM) in vanishing-twin pregnancies and (b) median values with 95% CI and *n* in vanishing-twin pregnancies (●), according to interval between embryonic demise and blood sampling, and in normal singleton pregnancies (◇). Dashed lines represent broken-stick model in vanishing-twin pregnancies. ES, empty gestational sac.

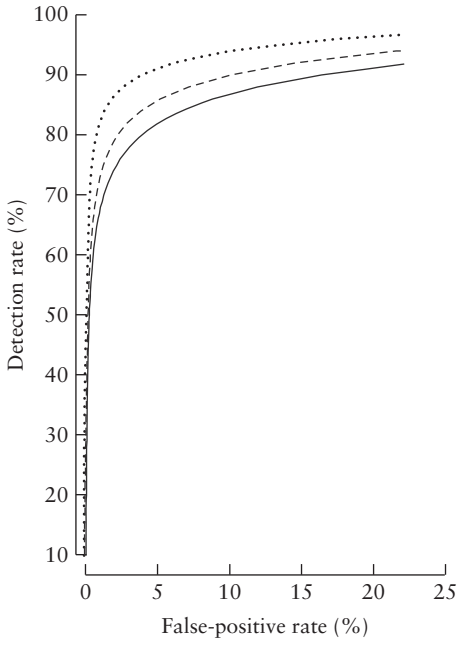

Figure 3 Receiver-operating-characteristics curves of predictive performance of first-trimester screening for trisomy 21 in singleton pregnancies, by combination of maternal age and fetal nuchal translucency thickness (NT) (——), maternal age, fetal NT and serum free beta-human chorionic gonadotropin (β-hCG) (– – –), and maternal age, fetal NT, serum free β-hCG and pregnancy-associated plasma protein-A (·····).

twin was to blood sampling ($P < 0.0001$) (Figure 2). A broken-stick model was fitted to $\log_{10}$ PAPP-A MoM, whereby, in those vanishing-twin pregnancies with a dead embryo and time since demise < 36 days, $\log_{10}$ PAPP-A MoM $= 0.2042 - 0.004206 \times$ (time since demise in days); 95% CI for intercept and slope of 0.069 to 0.3391 and −0.0083 to −0.00016, respectively. For those vanishing-twin pregnancies with a dead embryo and time since demise ≥ 36 days or those with an empty gestational sac, $\log_{10}$ PAPP-A MoM = 0.05302 (95% CI, 0.0333–0.0728) (Figure 2).

Performance of first-trimester combined test

The ROC curves of the predictive performance of first-trimester screening for trisomy 21 in singleton pregnancies are shown in Figure 3. The estimated DR, at a 5% FPR, was 82% in screening by a combination of maternal age and fetal NT, and this increased to 86% with the addition of serum free β-hCG and to 91% with the addition of serum PAPP-A.

DISCUSSION

Main findings

First, in pregnancies with a vanishing twin, median free β-hCG MoM is not significantly different from that in normal singleton pregnancies and there is no significant change with interval between fetal demise and blood sampling. Second, PAPP-A MoM is higher in vanishing-twin pregnancies than in normal singleton pregnancies, both in the group with an empty gestational sac and in that with a dead embryo. Third, in vanishing-twin pregnancies with a dead embryo, PAPP-A MoM is related inversely to the interval between estimated gestational age at embryonic demise and blood sampling. Fourth, in first-trimester screening for trisomy 21 in singleton pregnancies, the estimated DR, at a 5% FPR, is 82% in screening by a combination of maternal age and fetal NT and this increases to 86% with the addition of serum free β-hCG and to 91% with the addition of serum PAPP-A. Fifth, similar performance of screening can be achieved in pregnancies with a vanishing twin, provided the appropriate adjustments are made to the level of PAPP-A for the interval between estimated gestational age at embryonic demise and blood sampling.

Our findings confirm that clearance of free β-hCG from the maternal circulation is very rapid, whereas that of PAPP-A is slow. Studies in multifetal pregnancies undergoing embryo reduction have reported that the levels of free β-hCG decrease very quickly, whereas those of PAPP-A persist for at least 8 weeks after reduction[23,24].

Comparison with findings of previous studies

We found that, in vanishing twins, serum free β-hCG MoM is not altered and PAPP-A MoM is increased both in those with a dead embryo and in those with an empty gestational sac. Four previous smaller studies on maternal serum free β-hCG and PAPP-A in pregnancies with a vanishing twin reported contradictory results. Chasen *et al.* examined 41 cases of a vanishing twin and 4536 normal singleton pregnancies and reported that death of one twin within 28 days prior to blood sampling was associated with increased levels of both free β-hCG and PAPP-A[14]. However, the incidence of IVF conception was much higher in vanishing-twin pregnancies than in singleton controls (63% *vs* 3%) and no adjustment to the levels of metabolites was made for method of conception; in pregnancies conceived by IVF, compared to natural conceptions, free β-hCG is about 10% higher and PAPP-A is 10% lower. Gjerris *et al.* examined pregnancies conceived by IVF and compared free β-hCG and PAPP-A between 56 pregnancies with a vanishing twin and 897 singleton pregnancies; they reported no significant differences in MoM values of metabolites between the two types of pregnancy, irrespective of whether in the vanishing twins embryonic death occurred at < 9 or at 9–13 weeks' gestation[15]. However, the singleton median for PAPP-A MoM was 0.74, rather than the expected 1.0, casting doubt on whether the population gestation-specific medians had been optimized. Spencer *et al.* examined 270 cases of a vanishing twin and 1360 normal singleton pregnancies and reported no significant difference in MoM values of free β-hCG; PAPP-A levels were not altered in vanishing-twin pregnancies with an empty gestational sac, but, when the second gestational sac contained a dead embryo, PAPP-A was increased and the size of the increase was related to the time interval between fetal demise and blood sampling[16]. Huang *et al.* examined 137 cases of a vanishing twin and 683 normal singleton pregnancies and reported that MoM values of PAPP-A were increased in pregnancies with embryonic demise occurring at ≤ 28 days prior to blood sampling, but not in those with demise at > 28 days[17].

Strengths and weaknesses

In our study, which was considerably larger than the previous ones, the vanishing-twin and normal singleton pregnancies were matched for method of conception and there was optimization of free β-hCG and PAPP-A MoM values, both of which were 1.0 in the control group. A limitation of the study is that the interval between embryonic demise and blood sampling was based on the CRL of the dead embryo and it was assumed that this embryo was growing normally until the time of death, which is unlikely to be the case. Nevertheless, this is a pragmatic approach in the absence of serial ultrasound examinations.

The differential diagnosis of an empty gestational sac in the presence of another gestational sac with a living embryo at the 11–13-week scan includes vanishing twin, chorioamniotic separation and an old subchorionic collection following resolution of subchorionic hemorrhage. It is therefore possible that the diagnosis of a vanishing twin in some of our cases may not be true; however, this number is likely to be very small because 64% of our pregnancies were conceived through the use of assisted reproduction techniques and, in these cases, there was an early scan at 6–8 weeks' gestation and, additionally, in 63% of our cases, the 11–13-week scan demonstrated a dead embryo.

Implications for clinical practice

In vanishing twins, the option of screening by cfDNA testing of maternal blood is not available[11–13]. In relation to the first-trimester combined test, the UK National Health Service Fetal Anomaly Screening Programme recommends that, in vanishing-twin pregnancies with an empty gestational sac, the combined test can be used for screening because serum free β-hCG and PAPP-A are no different from those in singleton pregnancy; when the second sac has a detectable dead embryo, first-trimester screening should rely on fetal NT alone because maternal biochemical markers are increased for many weeks[14]. The document also claims that, in vanishing-twin pregnancies with an empty sac or a dead embryo, the biochemical markers used for second-trimester serum screening are not different from those in singleton pregnancy, and the quadruple test, composed of alpha-fetoprotein (AFP), unconjugated estriol, inhibin A and total hCG, can be used[25]. Our findings contradict the above recommendation in relation to first-trimester screening, and previous publications contradict the recommendation in relation to second-trimester screening. Abbas *et al.* reported that increased AFP levels persisted for at least 8 weeks following fetal reduction[26]. Huang *et al.* examined 154 cases of a vanishing twin, diagnosed at 11–13 weeks' gestation, and reported that MoM values of AFP at 15–19 weeks were increased by 10%; the study also examined inhibin-A levels in 40 pregnancies with a vanishing twin and found this to be increased by 13%[17].

Conclusions

First-trimester screening for trisomy in vanishing-twin pregnancies with an empty gestational sac or a dead embryo should rely on a combination of maternal age, fetal NT and serum free β-hCG, as in singleton pregnancy, without the use of serum PAPP-A. Alternatively, PAPP-A can be included but only after appropriate adjustment for the interval between estimated gestational age at fetal demise and blood sampling.

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