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First trimester aneuploidy screening in the presence of a vanishing twin: implications for maternal serum markers

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Objective To assess the impact of a vanishing twin on the levels of the biochemical markers used in the first trimester aneuploidy screening.

Methods A retrospective analysis of free β-hCG and PAPP-A levels in 270 women with a normal singleton fetus with ultrasound evidence of a vanishing twin pregnancy. Marker levels (as MoM) were compared in three groups—76 women with a second empty gestational sac, 194 women with a second gestational sac containing a dead fetus with a measurable crown rump length (CRL), and 1360 matched singleton pregnancies.

Results In women with a second empty gestational sac, the median free β-hCG and PAPP-A MoMs (0.968 and 1.040, respectively) were not significantly different from the 1.0 MoM in singleton pregnancies. In the group with a vanished twin with a measurable—CRL—there was a significantly increased median PAPP-A MoM (1.317) but the median free β-hCG MoM was not changed (1.024). Modelling this bias in PAPP-A MoM the detection rate for trisomy 21 would fall from 85 to 75%.

Conclusion First trimester screening in the presence of a vanishing twin may lead to errors in risk estimation. In such circumstances it may be advisable to restrict screening to the use of nuchal translucency (NT) alone.

KEY WORDS: Down syndrome; prenatal screening; PAPP-A; free β-hCG; trisomy; vanishing twin

INTRODUCTION

First trimester screening which combines the ultrasound measurement of nuchal translucency (NT) with the biochemical measurement of maternal serum free β-hCG and PAPP-A is one of the most effective methods of screening for Down syndrome (trisomy 21) enabling detection rates of 90% to be achieved at false-positive rate of 5% or less (Spencer *et al*., 1999; Nicolaides *et al*., 2005). In addition this method of screening also identifies a large proportion of the other major aneuploidies such as trisomies 13 and 18 (Tul *et al*., 1999; Spencer *et al*., 2000b), triploidy (Spencer *et al*., 2000d), and various sex aneuploidies (Spencer *et al*., 2000a).

A number of confounding factors are known to influence primarily the maternal serum biochemical markers. These include gestational age, maternal weight, ethnicity, smoking status, parity, mode of conception in vitro fertilisation (IVF), twin pregnancy, and chorionicity (Spencer, 2000, 2005; Spencer *et al*., 2000c,f, 2003, 2004, 2005a, 2008; Liao *et al*., 2001; Kagan *et al*., 2007, 2008). Most of these confounders have such a significant influence as to require correction for. Others such as diabetes, fetal sex, previous aneuploidy history, early vaginal bleeding (Cuckle *et al*., 2005; Cowans *et al*., 2009; Spencer *et al*., 2000e, 2005b, 2009a,b) and previous screening result (Spencer, 2001, 2002) are either too small or too problematical to make correction for.

The value of screening in twins using combined screening has been established in some centres where the use of specific twin correction and chorionicity correction can allow the identification of approximately 80% of twins discordant for trisomy 21 (Spencer and Nicolaides, 2003; Spencer *et al*., 2008). In early twin pregnancies, spontaneous reduction of a twin to a singleton pregnancy is a frequent occurrence with figures quoted between 10 and 40% (Landy and Keith, 1998; Dickey *et al*., 2002). With the increased use of IVF the potential for this 'vanishing twin' phenomenon to have a significant impact on screening has not been much studied. While there appears to be an association between the demise of a co-twin and vaginal bleeding (Saidi, 1988; De Sutter *et al*., 2006) there are no clinical symptoms in most cases (Jauniaux *et al*., 1988). Further more a recent extensive study has confirmed that early vaginal bleeding does not impact on maternal serum biochemical marker levels in the first trimester (Spencer *et al*., 2009b). Only two studies have examined the impact of a vanishing twin on the biochemical markers used in first trimester combined screening (Chasen *et al*., 2006; Gjerris *et al*., 2009). The aim of our study was to evaluate in a large cohort of women screened in three related centre, whether the presence of an empty second fetal sac or the presence of a vanishing twin has any impact on the level of the biochemical markers used in first trimester screening.

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METHODS

Information on vaginal bleeding at any time in pregnancy prior to screening is routinely sought either on the first trimester screening request form or during the patients consultation in the screening centres at King George Hospital, Goodmayes (formerly at Harold Wood Hospital, Romford) and at Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London and at the Fetal Medicine Centre, London. In all the three centres all relevant clinical information is recorded in a fetal database (PIA-Fetal Database, ViewPoint, Wessling, Germany) along with ultrasound information, ultrasound observations and the biochemical parameters measured as part of a first trimester screening program. At each of the centres ultrasound is carried out according to the Fetal Medicine Foundation (FMF) criteria by sonographers who have fulfilled the certificate of competency in the first trimester scan and crown rump length (CRL) and NT are measured in a standardised way (www.Fetalmedicine.com). At King George Hospital and at The Fetal Medicine Centre the biochemical markers were measured by time resolved amplified cryptate emission using the Brahms Kryptor system (Brahms AG, Hennigsdorf, Germany) while at King's College the markers were measured using time resolved fluorescence using the DelfiaXpress system (PerkinElmer Life Sciences, Turku, Finland).

A query of the databases at the three sites was set-up to extract relevant data in all live born singleton pregnancies unaffected by aneuploidy which had been screened and in which there was either ultrasound evidence of an empty second gestational sac or a second sac containing a fetus with a measurable crown rump length (CRL) but no fetal heart beat. For comparison a control group of singleton pregnancies were selected by taking five controls per case taken from within the same screening period as the cases. The measured concentration of each of the analytes free β-hCG and PAPP-A where converted to MoMs using the gestation as estimated from CRL and corrected for gestational age, maternal weight, ethnicity, parity, smoking status, mode of conception (IVF) (Spencer, 2000, 2005; Spencer *et al*., 2000c,f, 2003, 2004, 2005a, 2008; Kagan *et al*., 2007; Liao *et al*., 2001), and machine according to the FMF 2007/8 algorithm (Kagan *et al*., 2008).

Free β-hCG and PAPP-A MoMs were compared between the control group and those with an empty gestational sac using nonparametric statistics (median tests and Mann–Whitney test) and by t-tests on the log transformed medians. In the group with a vanishing twin with a measurable CRL—the marker MoMs were compared against the control group as described above. In addition the gestational age at fetal demise of the vanishing twin was calculated from Robinson and Fleming (1975) and the difference between the gestational age of blood sampling and fetal demise of the vanishing twin was calculated. A further analysis was made between marker levels in those with a vanishing twin with fetal demise within 28 days of blood sampling (late vanishing twin) and those with a blood sample taken more than 28 days after fetal demise of the vanishing twin (early vanishing twin). Levels of statistical significance were set at $p < 0.05$.

For comparison purposes we used twin biochemical markers data from our previous article analysing levels in mono- and dichorionic twins (Spencer *et al*., 2008).

Monte Carlo simulation was used to asses the impact on detection rate of any deviation in median marker levels. Briefly likelihood ratios were computed from the previously published distributions (Kagan *et al*., 2008) and adjusted for measured bias in either the free β-hCG MoM or the PAPP-A MoM and used with maternal age to produce patient-specific risks for each case. Crude detection rates and false-positive rates were calculated by taking the proportions with risks above a given risk threshold. Maternal age-specific detection and false-positive rates were then produced and adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000–2002.

RESULTS

Table 1 summarises the three study group populations from the three centres. The Fetal Medicine Centre (FMC) is a private centre with a higher proportion of Caucasian nonsmoking women of an older age group and a higher IVF conception while the other two populations are routine NHS screened populations with a wider range of ethnic mix and a higher level of smokers.

Table 1—Summary of the study population

Variable	All vanishing twin	Empty gestational sac	Singleton controls
Number	193	76	1361
% KGH	26.9	32.9	27.6
% FMC	59.6	53.9	58.7
% KCL	14.0	13.2	13.7
% Loss >28 days	64.8		
% Loss 1–28 days	35.2		
Median maternal age (years)	36.3	37.8	36.5
Median maternal weight (kg)	64.5	60.4	64.0
Median GA (days)	86.0	86.0	87.0
Median CRL (mm)	61.0	59.3	62.8
% IVF	53.4	59.2	38.5
% Ovulatory drugs	5.2	5.3	7.6
% Spontaneous	41.4	35.5	53.9
% Nulliparous	88.1	85.5	51.7
% Smoker	1.6	6.6	2.5
Ethnicity			
% Caucassian	91.2	94.8	89.2
% Afro-Caribbean	2.6	1.3	3.3
% Asian	4.1	2.6	5.4
% Oriental	0	0	1.0
% Other	2.1	1.3	1.1

KGH, King George Hospital; FMC, Fetal Medicine Centre; KCL, King's College Hospital; GA, gestational age

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Table 2—Summary of results in vanishing twins, singleton controls and previously published data on mono- and dichorionic twins[a] (Spencer *et al*., 2008)

Variable	All vanishing twins	Empty twin sac	Singleton controls	Dichorionic twins[a]	Monochorionic twins[a]
Number	193	76	1361	1024	190
Median-free ß-hCG MoM	1.024	0.968	0.983	2.041	1.983
Median PAPP-A MoM	1.317	1.040	0.964	2.250	1.756

[a] Data as published in Spencer *et al*., 2003

The proportion of assisted conception pregnancies in the vanishing twin and empty gestational sac groups was larger than the control group as was the incidence of nulliparity—these differences will have no impact on the biochemical marker levels because the medians have already been corrected for the impact these factors have on biochemical marker levels. Similarly the proportion of smokers in the empty gestational sac group is higher than in the control population—but again this factor is already accounted for in calculating the MoMs.

The overall median MoM free β-hCG was 0.983 in 1360 controls. The median MoM in those 76 women with an empty second gestational sac was 0.968 and this difference was not statistically significant ($p = 0.8677$). The overall median MoM PAPP-A was 0.964 in the 1360 controls. The median MoM in those women with an empty gestational sac was 1.040, this difference was not statistically significant ($p = 0.1290$).

In the group of women with a vanishing twin the overall median MoM free β-hCG was 1.024 and this was not significantly different to the 0.983 in the control group ($p = 0.7182$). The overall median MoM PAPP-A in this group was 1.317 and this was significantly higher than 0.964 in the control group ($p < 0.0001$). Of the vanishing twin cases 126 had suffered fetal demise more than 28 days (early vanishing twin) prior to the biochemical test and 68 had suffered fetal demise between 1 and 28 days (late vanishing twin) prior to the test. The median free β-hCG in the early vanishing twin group was 0.955 and was not significantly different ($p = 0.7922$) from controls while that in the late vanishing twin group was 1.154 and this did not reach significance ($p = 1.063$). The median PAPP-A MoM in the early vanishing twin group was 1.186 and was significantly increased ($p < 0.0001$) compared to controls while that in the late vanishing twin group was 1.543 and was also significantly increased ($p < 0.0001$). Table 2 summarises the results in the case of vanishing twins (with and without an empty sac) along side previously published data for monochorionic and dichorionic twins.

When we examined the trend in MoM by the time difference between demise of vanishing twin and the blood test we found no significant correlation for free β-hCG log MoM (Spearman $r_s = -0.12$, $p = 0.0996$) as shown in Figure 1. However, for PAPP-A there was a significant correlation of time between events and log PAPP-A MoM (Spearman $r_s = -0.24$, $p = 0.0008$) as shown in Figure 2.

When we evaluated the impact of the raised PAPP-A MoM on modelled screening performance we found that in the group of late vanishing twins the detection rate for trisomy 21 would fall from 85 to 75% at a risk cut-off of 1 : 100 (at sampling) with a consequent fall in

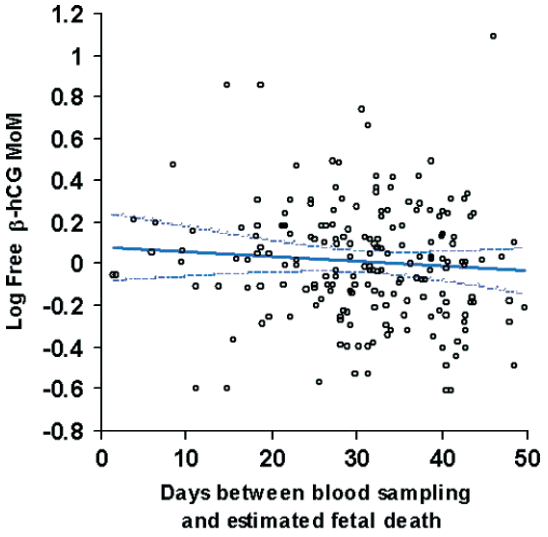

Figure 1—Correlation of log free β-hCG MoM with the difference in days between the calculated time of fetal demise and the sample collection date in cases with a vanishing twin. The solid line is the line of best fit ($y = 0.08482 - 0.002352x$) and the dotted line the 95% confidence interval

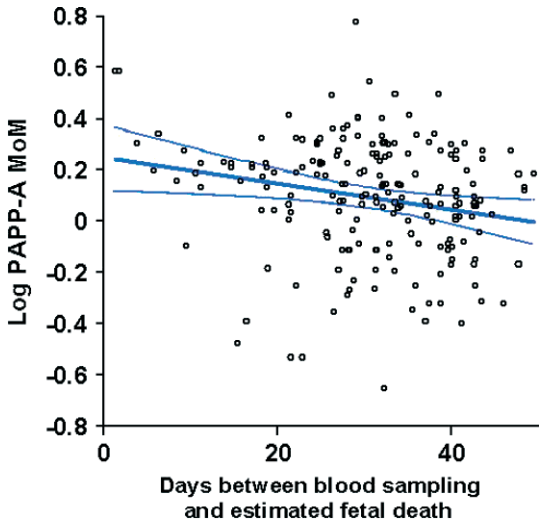

Figure 2—Correlation of log PAPP-A MoM with the difference in days between the calculated time of fetal demise and the sample collection date in cases with a vanishing twin. The solid line is the line of best fit ($y = 0.2479 - 0.005094x$) and the dotted line the 95% confidence interval

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false-positive rate from 2.6 to 0.8%. For cases of early vanishing twin the detection rate was 81% with a 1.6% false-positive rate.

DISCUSSION

The results of this study have shown that in the presence of an empty second gestational sac biochemical marker levels of free β-hCG and PAPP-A are not different to those in singleton pregnancies and therefore risks can be calculated as if a singleton pregnancy. When the second gestational sac contains a dead fetus the biochemical marker levels of free β-hCG are very similar to those in a singleton pregnancy but the levels of PAPP-A are increased and the size of the increase is related to the time interval between fetal demise and blood sampling. Thus, in instances of a vanishing twin—treating the PAPP-A result as if it came from a singleton pregnancy will result in an error in the risk estimation, such that potentially a reduction in detection rate may be seen. The size of this error will depend upon how recent the fetal demise was, and in such circumstances, in the interest of providing the best estimate of risk for the couple, it may be advisable to use NT or NT and free β-hCG only in the estimate of risk.

Two previous studies have looked at the issue of biochemical markers in the presence of a vanished twin. Chasen *et al.* (2006) in a study of 41 cases of vanishing twins found that spontaneous reduction was not associated with different levels of either analyte overall but did find that spontaneous reduction within 4 weeks of blood sampling did result in higher PAPP-A (1.79 vs 1.18, $p = 0.002$) and higher free β-hCG (1.28 vs 0.96, $p = 0.03$) when compared with normal singleton pregnancies. One of the criticisms of this study was that the group with a vanishing twin were 64% IVF pregnancies and the marker levels had not been corrected for IVF—although since IVF tends to result in a small increase in free β-hCG (which may have explained the significance in the late vanishing twin group) with PAPP-A IVF results in a small reduction in levels which obviously does not explain any increase seen in the cases of vanishing twins.

In a recent study, Gjerris *et al.* (2009) compared biochemical marker levels in 56 ART pregnancies with a vanishing twin with 897 ART singleton pregnancies. They could find no significant differences in geometric mean MoM for either free β-hCG or PAPP-A between pregnancies with an early (before 9 weeks) or late vanishing twin (9–13 weeks) or singleton pregnancies—although the overall singleton median for PAPP-A was 0.77, much less than the 1.00 expected—casting doubt on whether the population gestation specific medians had been optimised. Despite showing no difference in marker levels between early and late vanishing twins and vanishing twins, overall this study recommended that in early vanishing twins treating the pregnancy as a singleton for risk calculation was recommended—but in late vanishing twins it was advisable to base the risk assessment on ultrasound only.

In contrast therefore our extensive study has not only shown the impact of a vanishing twin to have no impact on levels of the free β-hCG marker but rather a large and significant increase in PAPP-A is related to the time between fetal demise and blood sampling and that this will have a significant impact on risk assessment in such cases.

It is not surprising that fetal demise has very little impact on free β-hCG levels in pregnancies with a vanishing twin. Free β-hCG is primarily cleared via the renal route and, under conditions of normal renal function (Spencer *et al.*, 2009c), has a very rapid clearance rate. Studies in nonpregnant individuals using radioactive intact hCG and the individual subunits have shown that the clearance is bi-exponential with an initial disappearance half-life of 0.68 h and a slower disappearance half-life of 3.93 h (Wehmann and Nisula, 1981, 1980, 1979) for free β-hCG which is much greater in the case of intact hCG (6 and 35.6 h). More recently Korhonen *et al.* (1997) studied the disappearance rates of intact hCG and its subunits after term pregnancy and concluded that the disappearance rates fitted a three component exponential model better than the two component model proposed by Wehman and Nisula and that the clearance half-lives for hCG were longer than previously estimated in the nonpregnant state, being 3.6, 18.0, and 53.0 h for intact hCG and 0.6, 6.2, and 21.9 h for free β-hCG. However, this increased estimated clearance time was at term and there is some evidence certainly for other markers that clearance in the first trimester is significantly shorter than at term. On this basis, assuming the earlier estimates of Wehman and Nisula to be most relevant at around 9–13 weeks, then any free β-hCG produced from any residual placental tissue from the perished embryo will have been cleared very rapidly—in the space of 24 h more than six half-lives will see a reduction to the normal singleton level of free β-hCG. Further, evidence to support this comes from studies of markers in the case of embryo reduction in multifetal pregnancies, when twin pregnancies have been reduced to singleton pregnancies during the first or early second trimester hCG levels returned to singleton levels very quickly (Johnson *et al.*, 1994).

Lin *et al.* (1976) showed that PAPP-A remains detectable in the maternal circulation 4–6 weeks postpartum. Smith *et al.* (1979) initially showed that the clearance half-life of PAPP-A after delivery at term was 51 h. This was confirmed by Bischof *et al.* (1984) who examined the clearance rate of PAPP-A at the end of normal pregnancy and after first trimester termination and found that PAPP-A also has a two component exponential clearance model. At term the initial clearance half-life was 52.9 h and the second phase clearance half-life was 142.9 h, considerably longer than for free β-hCG. However, when they examined this in women having a first trimester abortion the initial clearance half-life was 93.9 h and the second phase clearance half-life was 362.9 h. Thus, in the first trimester PAPP-A has a significantly extended half-life compared with many placental proteins which has led to the view that the trophoblast and the deciduas may not be the only source

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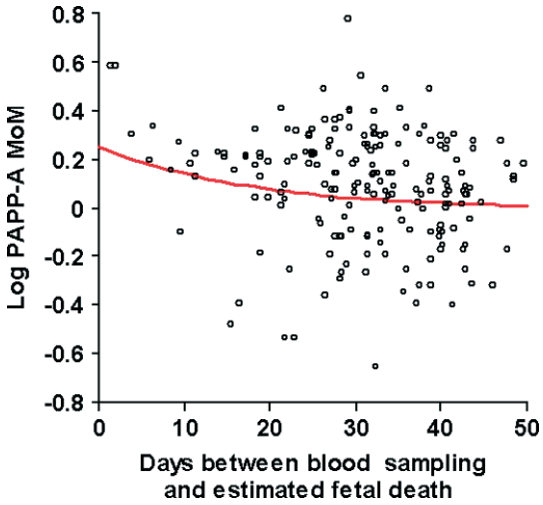

Figure 3—Log PAPP-A MoM with the difference in days between the calculated time of fetal demise and the sample collection date in cases with a vanishing twin. Solid line represents the second phase clearance half-life decay curve

of PAPP-A or that its clearance is via a more circuitous route.

The consequences for the extended half-life of PAPP-A in the situation of a vanishing twin is that PAPP-A remains at high levels even after fetal demise. Data from early studies on fetal reduction in multifetal pregnancies also confirmed that PAPP-A levels remain elevated at twin-like levels even 8 weeks after reduction (Abbas *et al*., 1996). When we plotted the second phase clearance half-life against the PAPP-A data from the cases with a vanishing twin and the time between blood sampling and fetal demise the disappearance curve (Figure 3) closely followed the data in the cases.

In conclusion therefore in the presence of an empty gestational sac it is reasonable and accurate to calculate the individual patient-specific risk as a singleton pregnancy and in such circumstances the marker levels appear to behave as those of a singleton. In the case of a vanishing twin while free β-hCG remains unaffected by the demise of the vanished twin, PAPP-A levels are significantly elevated and the level of the elevation is related to the time interval between fetal demise and blood sampling and that this is related to the extended clearance half-life of PAPP-A in the first trimester. Under such circumstances treating such pregnancies a singleton pregnancies will result in a positive biased PAPP-A result which in itself could lead to potential loss of detection of cases. It is advisable therefore in the presence of a vanished twin to either use NT and free β-hCG in the risk algorithm and exclude PAPP-A or perhaps more simply to rely on ultrasound NT alone.

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