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First-trimester maternal serum PAPP-A, SP1 and M-CSF levels in normal and trisomic twin pregnancies

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Objective To study PAPP-A and SP1 for biochemical trisomy screening in twin pregnancies and to investigate the role of maternal and placental compartments in marker production by comparing the levels of the decidual cytokine M-CSF with the PAPP-A and SP1 from the placenta.

Methods Thirteen twin pregnancies with at least one chromosomally abnormal fetus were compared with 68 normal twin pregnancies. Sera were obtained between 11 + 3 and 13 + 6 weeks of gestation, and PAPP-A, SP1 and M-CSF levels were determined by immunoassay. These concentrations were also compared with gestation-matched groups of 18 singleton normal pregnancies and 18 singleton Down syndrome pregnancies.

Results PAPP-A and SP1, but not M-CSF, levels were higher in normal twin pregnancy than in normal singleton pregnancy. SP1 levels, but not PAPP-A, correlated to M-CSF. PAPP-A, but not SP1, levels were reduced in abnormal twin pregnancies, with an increasing effect according to the number of affected fetuses, and were more pronounced in pregnancies with trisomy 18 or 13 than in trisomy 21 fetuses. M-CSF was inconsistent, with a trend towards increased levels in trisomy 21.

Conclusion PAPP-A remains the best biochemical screening marker for fetal trisomies 21, 18 or 13, in singleton as well as in twin pregnancy. In contrast to SP1, its site of production is not likely to be restricted to the placenta. The role of the (maternally produced) M-CSF remains to be further investigated.

KEY WORDS: twin pregnancy; first-trimester biochemical trisomy screening; PAPP-A

INTRODUCTION

Pregnancy-associated plasma protein A (PAPP-A) and pregnancy-specific β1-glycoprotein (SP1) are large glycoproteins (720 and 90 kDa, respectively) of placental origin and were first described three decades ago (Lin *et al*., 1974; Tatarinov and Masyukevich, 1970, respectively; for a review see Bischof, 1984). Maternal serum concentrations of both proteins show a continuous increase during pregnancy (Folkersen *et al*., 1981; Towler *et al*., 1976). PAPP-A levels were found to be reduced in the first trimester of pregnancies affected by fetal trisomy 21 (T21, Down syndrome), which is the most frequent chromosomal abnormality (Wald *et al*., 1992; Muller *et al*., 1993; Brambati *et al*., 1994). We have confirmed this observation and found that SP1 was also reduced in T21 pregnancies compared to gestation-matched controls, but to a lesser extent than PAPP-A and in correlation to it (Bersinger *et al*., 1994). Since then, first-trimester biochemical T21 screening has been established on the basis of PAPP-A and the free beta subunit of human chorionic gonadotropin (Fβ-hCG) (Wheeler and Sinosich, 1998; Wald and Hackshaw, 2000); these two markers are independent of each other. In trisomy 18, the reduction in serum levels was more pronounced for both PAPP-A and SP1 in the first trimester (Bersinger *et al*., 1994; Brizot *et al*., 1995) and, for PAPP-A only, persisted into the second trimester (Bersinger *et al*., 1999), in contrast to trisomy 21 (Cuckle *et al*., 1992; Knight *et al*., 1993).

Macrophage-colony-stimulating factor (M-CSF) is a 85-kDa glycoprotein cytokine, which in pregnancy is produced by the decidua, yielding increased serum levels over non-pregnant subjects (Tsakonas *et al*., 1995). M-CSF may play a role in placental maintenance as its serum levels were found to be reduced in recurrent aborters (Katano *et al*., 1997) and increased in pre-eclampsia (Hayashi *et al*., 1996); moreover it was found to stimulate trophoblastic hCG production *in vitro* (Saito *et al*., 1991). With the placental compensatory mechanisms operating in T21 (Bersinger *et al*., 1995a) and other fetoplacental abnormalities, we decided to investigate the role of M-CSF in Down syndrome pregnancies.

Placenta-derived markers, in a chromosomally normal pregnancy, show increased serum levels in the presence of a higher number of fetuses. Thus, the normal medians (obtained from singleton pregnancies) cannot be used for screening twin pregnancies. With a growing interest in first-trimester testing, medians for Fβ-hCG have been calculated for twin pregnancies but this marker did not yield satisfactory results in a report with 10 single and 2 double T21 cases (Noble *et al*., 1997). When ultrasound analysis (nuchal translucency thickness) is included, a high T21 detection rate can

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be reached in twins (Spencer, 2000). In this biochemical study, with PAPP-A being superior to Fβ-hCG in singleton T21 pregnancies and the Fβ-hCG results in twins, we decided to investigate the placental proteins PAPP-A and SP1 in twin pregnancies, together with M-CSF.

PATIENTS, MATERIALS AND METHODS

Sera from 13 twin pregnancies with abnormal (trisomic) fetal karyotype were available for this retrospective study. The gestational age range was $11+3$ to $13+6$ (average, $12+5$) weeks. The karyotypes were as follows: $1\times$T21/$1\times$Normal, $N=6$; $1\times$T18/$1\times$Normal, $N=2$; $2\times$T21, $N=3$; $1\times$T21/$1\times$T18, $N=1$; $2\times$T13, $N=1$. The control population consisted of 68 twin pregnancies of double-normal karyotype, covering the same gestational period (average, $12+4$ weeks). Serum was collected after centrifugation and stored at $-30\,^{\circ}$C in aliquots until analysis was performed in batches. PAPP-A and SP1 were both assayed by double-antibody microplate enzyme immunoassay (ELISA) methods developed in our laboratory. Polyclonal rabbit antibodies and their horseradish peroxidase conjugates were obtained from Dako (Denmark); the capture antibody for PAPP-A was further purified by negative affinity chromatography (Bersinger *et al*., 1995b) before use. Intra- and inter-assay coefficients of variance (CVs) were 4.5 and 9.7%, respectively. SP1 was determined similarly and without antibody pretreatment as described previously (Bersinger *et al*., 1995c); CVs for SP1 were 4.1 (intra-assay) and 8.7% (inter-assay), respectively. Calibration in both PAPP-A and SP1 tests was against the WHO reference preparation IRP-78-610. M-CSF was determined using a Quantikine® ELISA kit obtained from R&D Systems, Europe (UK) and following the manufacturer's instructions. A serum dilution of 1 : 3 was used and the incubation temperature was $30\,^{\circ}$C throughout. The results were log-transformed and the 13 twin trisomy cases were individually compared to the gestational age-dependent medians from the 68 normal twin pregnancies. Normal and trisomic twins were also compared with the medians from normal and trisomic singleton pregnancies. An in-house control singleton-pregnancy database yielded a PAPP-A median of 4.665 mIU/mL ($N=397$) and an SP1 median of 14.97 μg/mL ($N=60$) in the same gestational age range window. No M-CSF data were available from this source. Thus, 18 new singleton T21 and 18 gestation-matched singleton control samples, collected during the same period and analysed using the same assay protocols, were included in the study for the purpose of additional control. Medians were calculated from the groups with normal karyotype using log transformation and linear regression; these medians were used to calculate the individual multiples of median MoMs in the affected cases. Group analysis was done with the non-parametric Mann–Whitney U test. A possible connection between M-CSF and the production of placental proteins was investigated by linear regression analysis for these markers in chromosomally normal twin and singleton pregnancies.

RESULTS

In the control twin-pregnancy population, all three markers PAPP-A, SP1 and M-CSF were significantly dependent on the gestational age (Figure 1, left panels), with the strongest increase in serum levels observed for PAPP-A ($r=0.5328$, $p<0.0001$, Figure 1A). This increase (log-slope = 0.239) was also steeper than the one observed in our normal singleton-pregnancy controls over the same interval of gestational age (log-slope = 0.078, Figure 2A), or the one calculated from our pre-existing database (log-slope = 0.105). These slopes correspond to the concentration doubling times of 1.26, 3.86 and 2.93 weeks, respectively, for PAPP-A in twins, singleton-current and singleton-database pregnancies, or increases of 73, 20 and 27% per gestational week for PAPP-A and 23, 14 and 24% for SP1. PAPP-A and SP1 also showed increased absolute median levels in twin compared to singleton (both current and pre-existing set) pregnancies; this was not the case for M-CSF.

Twelve out of the 13 cases affected by single or double fetal trisomy had a reduced level of PAPP-A, with the double trisomies showing a clearly more pronounced effect (Figure 1A, right panel). Individual MoM values for the three markers are listed for the 13 cases in Table 1. The presence of one or two Down syndrome (T21) fetuses did not seem to affect SP1 levels while the T18/T13 cases tended to yield reduced levels (Figure 1B). M-CSF tended to be increased in T21 and unaffected in the other cases (Figure 1C). Analysis in groups was performed for trisomy 21 cases only (there were too few of the other trisomies), and the results are presented in Table 2 (this was possible since there was no difference in the mean gestational age). When compared with normal twin pregnancy, PAPP-A MoMs for pregnancies with one Down syndrome fetus (and one normal), or with two affected fetuses were 0.77 ($p=0.230$) and 0.64 ($p=0.051$), respectively. This

Table 1—Multiples of median (MoMs) for PAPP-A, SP1 and M-CSF in the 13 twin pregnancies presenting single or double fetal chromosomal abnormalities

Patient ID	Gestation at sampling (weeks)	Karyotype (Trisomies)	Multiples of median (MoM) PAPP-A	SP1	M-CSF
44094	$11+5$	1xT21	0.517	0.395	2.686
46321	$12+0$	1xT21	0.907	1.629	1.262
21984	$12+0$	1xT21	1.134	1.710	1.389
24759	$12+4$	1xT21	0.685	1.871	9.444
36449	$13+1$	1xT21	0.701	1.703	0.669
28845	$13+6$	1xT21	0.678	0.602	1.994
31569	$12+3$	1xT18	0.330	0.570	0.921
44053	$13+3$	1xT18	0.961	0.543	0.592
1426	$13+6$	1xT21, 1xT18	0.265	1.705	0.886
39424	$11+4$	2xT21	0.207	1.088	0.813
38919	$12+3$	2xT21	0.681	1.326	1.115
32097	$13+5$	2xT21	0.415	0.850	1.578
42155	$12+4$	2xT13	0.252	0.555	1.195

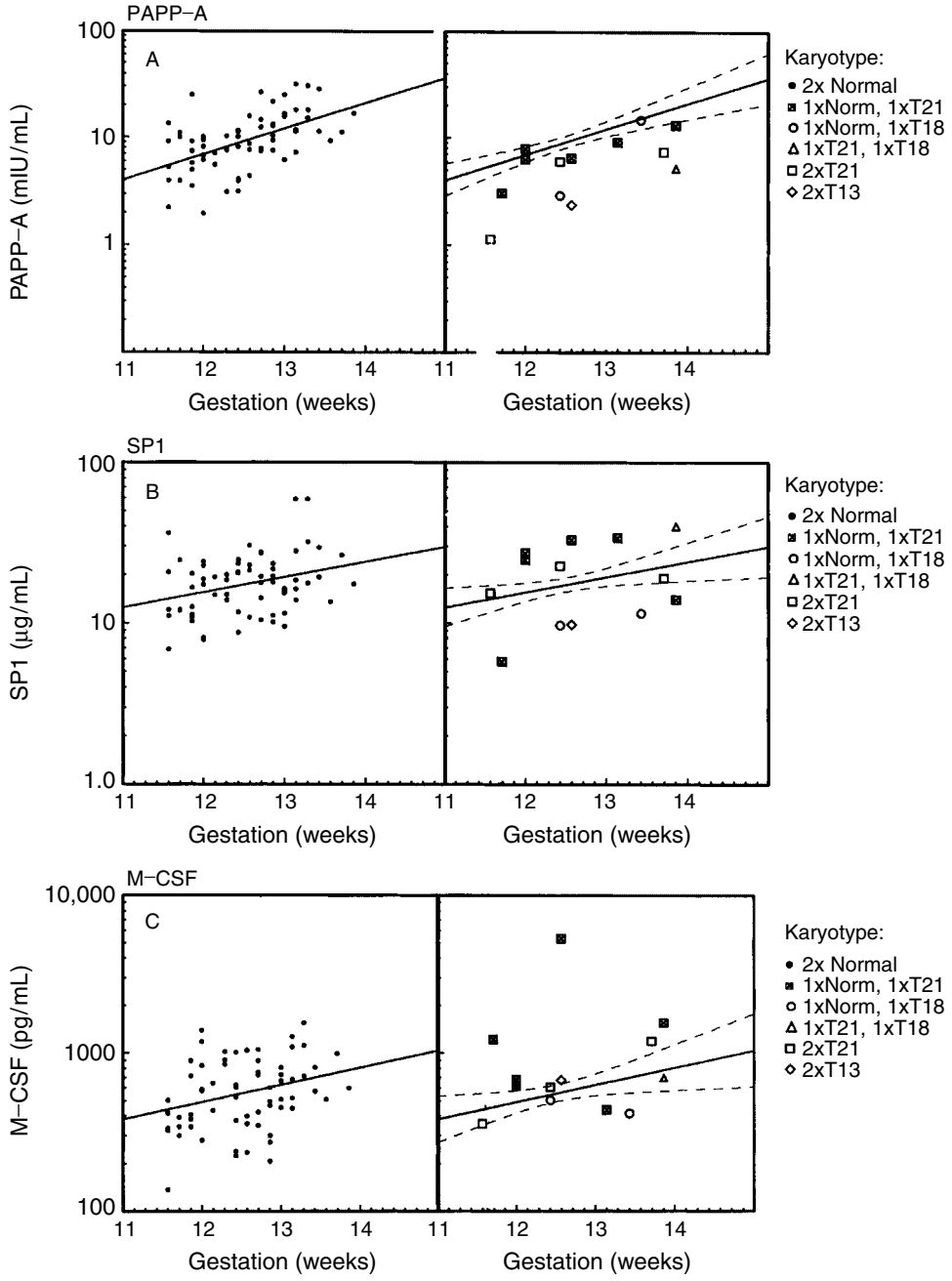

Figure 1—PAPP-A, SP1 and M-CSF in a chromosomally normal twin pregnancy (left panels) and in single or double fetal trisomies (right panels). Regression lines, after log transformation, are shown in both panels for the normal pregnancies, including the 95% confidence interval in the right panels. ●, normal karyotype; symbols for individual pathological karyotypes are shown beneath the graphs. (A) PAPP-A; (B) SP1; and (C) M-CSF

reduction was significant ($p = 0.041$) when single and double T21 cases were taken together ($N = 9$), but was not as pronounced as the one observed in singleton Down syndrome versus normal pregnancies (MoM = 0.47, $p = 0.0015$, $N = 18$, Figure 2A, right panel; this mean MoM was 0.51 when the pre-existing database of 397 singleton controls was used as a reference).

In the chromosomally normal pregnancies analysed here, the PAPP-A and SP1 levels were strongly correlated to each other (twins: $r = 0.583$, $p < 0.0001$, $N = 68$; singletons: $r = 0.602$, $p = 0.0083$, $N = 18$). Moreover, SP1, but not PAPP-A, was positively correlated to M-CSF in twins ($r = 0.341$, $p = 0.0044$) and after removal of one outlier in singletons ($r = 0.611$, $p = 0.0092$).

DISCUSSION

In this study, we have demonstrated and confirmed the presence of increased maternal serum levels of the

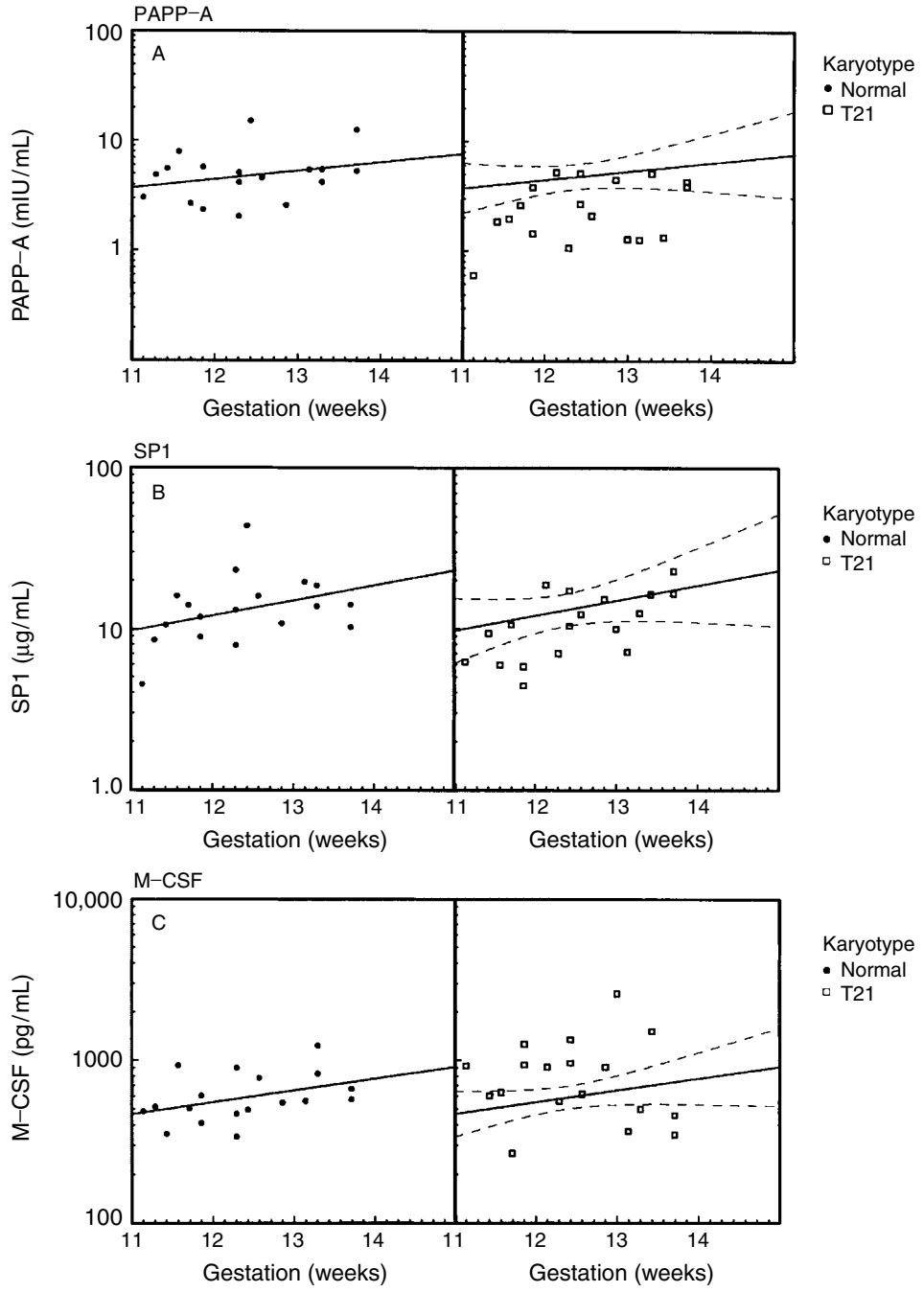

Figure 2—PAPP-A, SP1 and M-CSF in a singleton pregnancy with normal fetal karyotype (left panels) and with trisomy 21 (right panels). Regression lines, after log transformation, are shown in both panels for the normal pregnancies, including the 95% confidence interval in the right panels. ●, normal karyotype; ○, trisomy 21. (A) PAPP-A; (B) SP1; and (C)M-CSF

placental products PAPP-A and SP1 in twin compared to singleton pregnancies of matched gestational age; we found a PAPP-A MoM ratio of 1.87, which is the same as the one reported by Spencer (2000) but less than the value given in a more recent report (Niemimaa *et al*., 2002), which, with 2.36 was quoted to be higher than expected ($N = 67$). M-CSF, on the other hand, was not increased (Table 2), which is in agreement with its proposed maternal (decidual) origin (Tsakonas *et al*., 1995). Nevertheless, a correlation was found between the levels of SP1 and M-CSF in twins (and, to a lesser extent, in our smaller group of singletons that was only included in this study for control purposes), which goes alongside the observation that M-CSF could stimulate the production of hCG by cultured trophoblast cells (Saito *et al*., 1991). We were, however, unable to demonstrate a correlation between M-CSF and the other placental protein PAPP-A examined in this study. These findings favour the hypothesis that M-CSF exercises a specific stimulatory effect on the placenta that is

Table 2—Characteristics of twin- and singleton-pregnancy groups with respect to the presence of one or two Down syndrome (T21) fetuses

Karyotype	*N*	Gestational age (mean, SD) (weeks)	Median PAPP-A (mIU/mL)	Median SP1 (μg/mL)	Median M-CSF (pg/mL)
Twin pregnancies:					
2xNormal	68	12.5 ± 0.6	9.36	17.86	575
1xN/1xT21	6	12.5 ± 0.8	7.22[a]	25.98[a]	951[b]
2xT21	3	12.6 ± 1.1	6.01[a]	19.16[a]	609[a]
Singleton pregnancies:					
Normal	18	12.4 ± 0.8	5.00	13.08	554
T21	18	12.5 ± 0.8	2.37[c]	10.47[a]	768[a]

[a] $p > 0.05$ (non-significant) compared to the unaffected population with the same number of fetuses.
[b] $p = 0.046$ compared to normal twins.
[c] $p = 0.0015$ compared to normal singletons.

the sole source of hCG and SP1; PAPP-A, on the other hand, is not only produced in pregnancy by the placenta, but other sources could be involved, which is in agreement with previously reported observations (Bersinger *et al*., 1997). Nevertheless, PAPP-A and SP1 were strongly correlated in our group of twin pregnancies, as was the case in the singletons here and those previously reported (Bersinger *et al*., 1994); this illustrates the role of placental contribution in PAPP-A production.

PAPP-A was clearly reduced in trisomy 21 pregnancies: strongly in the singletons as well as in the twins with two T21 fetuses, and less strongly in one normal and one affected fetus, but our number of cases are small in these groups. SP1 was not reduced in twin pregnancies with single or double T21; singleton T21-pregnancy SP1 was reduced without reaching significance. This is in disagreement with a previous observation (Bersinger *et al*., 1994); a possible explanation for this can be provided by the later positioning of the gestation range in this study (late first leading into early second trimester). SP1 in a singleton T21 pregnancy was indeed shown to be reduced in the first and increased in the second trimester (Aitken *et al*., 1994), which logically results in an intermediate period without possible discrimination (Bersinger *et al*., 1995a). Moreover, the number of singleton control pregnancies included in this study was small; when the pre-existing database was used for SP1 ($N = 60$), the reduction was more pronounced (mean MoM = 0.70 instead of 0.80).

M-CSF tended to be increased in twin and singleton T21 pregnancies; statistical significance, however, was only reached in a small group of 6 twin pregnancies with one T21 and one normal fetus. The proof of the usefulness of M-CSF determinations in fetal-trisomy screening thus requires further investigation with a larger number of cases. M-CSF may be interesting for studying the biological and immunological role of the maternal compartment, but it is unlikely for this protein to play a clinically important role in this context. With respect to the trisomies other than T21, again only PAPP-A could clearly detect them (Figure 1A); M-CSF did not tend to be increased, as was the case in trisomy 21 (Figure 1C).

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