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. Copyright © 2003 John Wiley & Sons, Ltd.

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., 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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follows: 1 = N was collected after centrifugation and stored at
13 + mally normal twin and singleton pregnancies.
ear regression analysis for these markers in chromoso-
production of placental proteins was investigated by lin-
U test. A possible connection between M-CSF and the
alysis was done with the non-parametric Mann–Whitney
these medians were used to calculate the individual mul-
control samples, collected during the same period and
new singleton T21 and 18 gestation-matched singleton
in the study for the purpose of additional control. Medi-
analysed using the same assay protocols, were included
control samples, obtained from Dako (Denmark); the capture antibody for
Microplate enzyme immunoassay (ELISA) methods
PAPP-A and SP1 were both assayed by double-antibody
PAPP-A and SP1 in twin pregnancies, together with
M-CSF. 

PATIENTS, MATERIALS AND METHODS
Sera from 13 twin pregnancies with abnormal (tri-
monic) fetal karyotype were available for this retrospec-
tive study. The gestational age range was 11 + 3 to
13 + 6 (average, 12 + 5) weeks. The karyotypes were as
follows: 1 × T21/1 × Normal, N = 6; 1 × T18/1 × Normal,
N = 2; 2 × T21, N = 3; 1 × T21/1 × T18, N = 1; 2 × 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 °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 antibod-
ies and their horseradish peroxidase conjugates were
obtained from Dako (Denmark); the capture antibody for
PAPP-A was further purified by negative affinity chro-
matography (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 previ-
ously (Bersinger et al., 1995c); CVs for SP1 were 4.1
(intra-assay) and 8.7% (inter-assay), respectively. Cali-
bration 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 °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. Medi-
ans were calculated from the groups with normal kary-
otype using log transformation and linear regression;
these medians were used to calculate the individual mul-
tiples 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 lin-
ear regression analysis for these markers in chromoso-
mally 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
appear to be different (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>
<thead>
<tr>
<th>Patient ID</th>
<th>Gestation at sampling (weeks)</th>
<th>Karyotype (Trisomies)</th>
<th>Multiples of median (MoM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44094</td>
<td>11 + 5</td>
<td>1xT21</td>
<td>PAPP-A: 0.517, SP1: 0.395, M-CSF: 2.686</td>
</tr>
<tr>
<td>46321</td>
<td>12 + 0</td>
<td>1xT21</td>
<td>PAPP-A: 0.907, SP1: 1.629, M-CSF: 1.262</td>
</tr>
<tr>
<td>42155</td>
<td>11 + 4</td>
<td>1xT21</td>
<td>PAPP-A: 1.134, SP1: 1.710, M-CSF: 1.389</td>
</tr>
<tr>
<td>36449</td>
<td>13 + 1</td>
<td>1xT21</td>
<td>PAPP-A: 0.685, SP1: 1.871, M-CSF: 9.444</td>
</tr>
<tr>
<td>35925</td>
<td>13 + 1</td>
<td>1xT21</td>
<td>PAPP-A: 0.701, SP1: 1.703, M-CSF: 0.669</td>
</tr>
<tr>
<td>28845</td>
<td>13 + 6</td>
<td>1xT21</td>
<td>PAPP-A: 0.678, SP1: 0.602, M-CSF: 1.994</td>
</tr>
<tr>
<td>31569</td>
<td>13 + 3</td>
<td>1xT18</td>
<td>PAPP-A: 0.330, SP1: 0.570, M-CSF: 0.921</td>
</tr>
<tr>
<td>44053</td>
<td>13 + 3</td>
<td>1xT18</td>
<td>PAPP-A: 0.961, SP1: 0.543, M-CSF: 0.592</td>
</tr>
<tr>
<td>1426</td>
<td>13 + 6</td>
<td>1xT21, 1xT18</td>
<td>PAPP-A: 0.265, SP1: 1.705, M-CSF: 0.886</td>
</tr>
<tr>
<td>39424</td>
<td>11 + 4</td>
<td>2xT21</td>
<td>PAPP-A: 0.207, SP1: 1.088, M-CSF: 0.813</td>
</tr>
<tr>
<td>38919</td>
<td>12 + 3</td>
<td>2xT21</td>
<td>PAPP-A: 0.681, SP1: 1.326, M-CSF: 1.115</td>
</tr>
<tr>
<td>32097</td>
<td>13 + 5</td>
<td>2xT21</td>
<td>PAPP-A: 0.415, SP1: 0.850, M-CSF: 1.578</td>
</tr>
<tr>
<td>42155</td>
<td>12 + 4</td>
<td>2xT13</td>
<td>PAPP-A: 0.252, SP1: 0.555, M-CSF: 1.195</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Gestational age (mean, SD) (weeks)</th>
<th>PAPP-A (mIU/mL)</th>
<th>SP1 (µg/mL)</th>
<th>M-CSF (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twin pregnancies:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2xNormal</td>
<td>12.5 ± 0.6</td>
<td>9.36</td>
<td>17.86</td>
<td>575</td>
</tr>
<tr>
<td>1xN/1xT21</td>
<td>12.5 ± 0.8</td>
<td>7.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>951&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2xT21</td>
<td>12.6 ± 1.1</td>
<td>6.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>609&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Singleton pregnancies:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>12.4 ± 0.8</td>
<td>5.00</td>
<td>13.08</td>
<td>554</td>
</tr>
<tr>
<td>T21</td>
<td>12.5 ± 0.8</td>
<td>2.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>768&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

was 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 is 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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