antibody or a mixture of monoclonal antibodies that recognize the light and heavy chains of FVIIa was similar to that determined by immunoelectrophoresis. However, the level of the FVIIa:ag, using the monoclonal antibody recognizing the specific epitope located in the three-dimensional structure near position 79, was remarkably low compared with QIEP or ELISA using a polyclonal antibody or a mixture of monoclonal antibodies that recognize the light and heavy chains of FVIIa. The levels of FVIIa:ag of the father and sister revealed a similar pattern to that of the propositus, although they were not as low. These findings strongly suggested that the mutation in the abnormal FVII of the propositus, her father, and her sister was in the structure near position 79 in the first EGF-like domain of human FVII. DNA sequencing revealed a G-to-A point mutation that was found at nucleotide position 6055 in exon 4 of the FVII gene. This produces a substitution in the CCG codon for Arg 79 in the first EGF-like domain such that it is changed to CAG. The mutation in the propositus, her father, and her sister was confirmed by restriction endonuclease digestion.

Clark et al. (18) reported that the first EGF-like domain of FVII is essential for binding TF, as analyzed by the reaction of monoclonal antibodies with amino acid residues 51–88 of the first EGF-like domain of human FVII, which was mapped with fusion protein fragments. Interaction between FVIIa and TF involves direct contact between TF, the first EGF-like domain of FVIIa, and the catalytic domain (19). O’Brien et al. (20) showed that the first EGF-like domain of FVII plays a key role in FVII complex formation with TF, as analyzed by surface plasmon resonance of the interaction between TF and recombinant FVII-R79Q. We reported (7) that the loss of charge associated with the substitution of Arg by Gln at position 79 in FVII Shinjo has a direct effect on the TF-binding site in this part of the first EGF-like domain of FVII. Recently, Banner et al. (21) determined the x-ray crystal structure of the complex of active site-inhibited FVIIa with subtilisin-treated soluble TF and showed that Arg 79 was close to Glu 24 and Gln 56 in the N-terminal domain of TF. The amino acid residues close to Arg 79 of FVIIa in the complex are conserved in human, rabbit, and bovine TFs. Why FVII with the substitution of Arg by Gln at position 79 gives different procoagulant activities using TF from various species is still unknown and will require additional studies. Our ELISA system can be used to check the abnormal FVII molecules that give different procoagulant activities using TF from various sources.

Serum Carcinoembryonic Antigen, Cancer Antigen 125, Cancer Antigen 15-3, Squamous Cell Carcinoma, and Tumor-associated Trypsin Inhibitor Concentrations during Healthy Pregnancy, Marie-Hélène Schlager, Jérôme Larghero, Bruno Cassinat, Marie-Elisabeth Toubert, Caroline Borschneck, and Jean-Didier Rain (Service de Médecine Nucléaire, Hôpital Saint-Louis, 75475 Paris Cedex 10, France), * author for correspondence: fax 33 (0)1 42 49 94 05, e-mail schlager@chu-stlouis.fr

In the management of cancer patients, tumor-associated antigens are measured in serum as noninvasive tests for relapse detection [reviewed in Ref. (1)]. The tests have limited specificity because the serum concentrations of the

References
antigens can be affected by pathophysiological conditions such as renal insufficiency, hepatic diseases, and inflammation (2). The temporal changes of tumor markers during pregnancy are not well documented. Although some have reported values grouped by trimester of pregnancy (3), longitudinal studies are rare.

We have studied the tumor markers CEA (carcinoembryonic antigen), CA (cancer antigen) 125, CA 15-3, SCC (squamous cell carcinoma), and TATI (tumor-associated trypsin inhibitor), which are widely used in gynecological tumors. CEA is expressed in many malignancies and is useful for the monitoring of colon, lung, and breast cancers (4). CA 125 and CA 15-3 belong to the family of carbohydrate antigens also named mucins (1). CA 125, which was identified by a monoclonal antibody raised to an ovarian carcinoma cell line (OC125), is used for the follow-up of nonmucinous ovarian cancer (1). It displays increased seric concentrations in cases of serous effusions (2). CA 15-3, which was identified by two monoclonal antibodies 115D8 and DF3, is the most widely used tumor marker for breast carcinoma (4). SCC (or Ta-4) is a tumor-associated antigen isolated from squamous cell carcinoma of uterine cervix (5) and is used for the follow-up of epidermoid tumors (cervical uterine carcinoma or epidermoid lung cancer) (6). TATI was isolated from the urine of an ovarian cancer patient (7). It is used for the follow-up of mucinous ovarian cancer (8) and other malignancies including cancers from the urogenital tract (9). Its synthesis is regulated like acute phase proteins (10).

The aim of our work was to determine the specificity of these tumor markers in uncomplicated pregnancy. We obtained four to nine serum samples from each of 12 healthy pregnant women at 6–40 weeks of amenorrhea. Each marker was measured in 76 samples. Informed consent was obtained from all patients. The procedures followed were in accordance with the institution’s responsible committee.

Serum samples were stored frozen at −20 °C. CEA and SCC were measured by microparticular enzyme immunoassay on an IMX analyzer (Abbott). CA 125 and CA 15-3 were measured by immunoradiometric assay with ELSA-CA 125 and ELSA-CA 15-3 (CIS Bio International). TATI was assayed by RIA (SPECTRIA TATI, Pharmacia). Between-run variation coefficients were <5% for CEA and SCC and between 7% and 10% for CA 125, CA 15-3, and TATI.

In serum samples from 100 healthy blood donors, the 95th percentile values were 3 μg/L for CEA, 40 kIU/L for CA 125, 1.6 μg/L for SCC, and 25 μg/L for TATI, and the 100th percentile for CA 15-3 was 30 kIU/L.

Tests for a linear trend were performed for the temporal evolution of the five markers CEA, SCC, CA 125, CA 15-3, and TATI for the 12 patients. We tested whether the following model could explain variation in the data for each patient: marker \( i = \beta_0 + (\beta_1 \times \text{week}) + \epsilon \), where marker \( i \) is the dependent variable, i.e., CEA, SCC, CA 125, CA 15-3, or TATI; \( \text{week} \) is the regressor variable; \( \beta_0 \) and \( \beta_1 \) are the unknown parameters; and \( \epsilon \) is the unknown error. The principle of least squares produced estimates of the unknown parameters \( \beta_0 \) and \( \beta_1 \). The SAS statistical package was used for the analysis (SAS, Inc.).

Table 1 displays the overall values of the five tumor markers by periods of 4 weeks of pregnancy. In 29 samples (7.6% of all measurements), one or more markers were increased. Our results thus confirm previous reports that tumor markers are usually within their reference intervals during pregnancy.

In 45 cases (of 60 curves), the evolution of tumor markers with time could not be fitted by a linear model of regression. In 15 cases coming from 9 women, the curves

<table>
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</thead>
<tbody>
<tr>
<td>CEA</td>
<td>Mean ± SD</td>
<td>1.9 ± 0.7</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.8</td>
<td>1.4 ± 0.8</td>
<td>1.2 ± 0.7</td>
<td>1.4 ± 1.0</td>
<td>1.5 ± 0.7</td>
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<tr>
<td></td>
<td>Range</td>
<td>1.4–2.4</td>
<td>0.8–1.6</td>
<td>0.6–3.5</td>
<td>0.6–2.8</td>
<td>0.4–2.8</td>
<td>0.5–3.4</td>
<td>0.7–3.3</td>
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<tr>
<td>SCC</td>
<td>Mean ± SD</td>
<td>1.6 ± 1.8</td>
<td>0.5 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.7 ± 0.5</td>
<td>1.0 ± 0.4</td>
<td>1.4 ± 1.1</td>
<td>1.2 ± 0.5</td>
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<tr>
<td></td>
<td>Range</td>
<td>0.3–2.9</td>
<td>0.3–0.9</td>
<td>0.2–1.0</td>
<td>0.1–2.2</td>
<td>0.6–1.5</td>
<td>0.6–4.3</td>
<td>0.5–2.2</td>
</tr>
<tr>
<td>CA 125</td>
<td>Mean ± SD</td>
<td>12.8 ± 6.9</td>
<td>18.7 ± 14.0</td>
<td>21.4 ± 15.9</td>
<td>19.9 ± 12.5</td>
<td>19.1 ± 7.8</td>
<td>22.3 ± 13.1</td>
<td>22.1 ± 12.3</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>7.95–17.7</td>
<td>6.1–41.5</td>
<td>4.4–63.5</td>
<td>6.5–54.3</td>
<td>7.6–38.0</td>
<td>6.1–51.3</td>
<td>6.2–49.3</td>
</tr>
<tr>
<td>CA 15-3</td>
<td>Mean ± SD</td>
<td>12.3 ± 1.5</td>
<td>9.3 ± 4.0</td>
<td>12.9 ± 4.2</td>
<td>14.1 ± 4.1</td>
<td>17.1 ± 4.7</td>
<td>16.5 ± 4.1</td>
<td>16.9 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>12.2–14.3</td>
<td>5.0–15.0</td>
<td>9.2–20.5</td>
<td>10.0–22.8</td>
<td>8.9–24.8</td>
<td>8.8–24.2</td>
<td>5.1–27.0</td>
</tr>
<tr>
<td>TATI</td>
<td>Mean ± SD</td>
<td>12.0 ± 1.4</td>
<td>11.8 ± 5.4</td>
<td>14.4 ± 7.2</td>
<td>16.9 ± 7.7</td>
<td>18.3 ± 11.0</td>
<td>20.6 ± 10.8</td>
<td>26.8 ± 18.5</td>
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<tr>
<td></td>
<td>Range</td>
<td>11.0–13.0</td>
<td>5.0–18.0</td>
<td>6.0–33.0</td>
<td>7.0–35.0</td>
<td>9.0–44.0</td>
<td>7.0–44.0</td>
<td>9.0–65.0</td>
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</table>

n is the number of samples. Mean ± SD, expressed in μg/L for CEA, SCC, and TATI and in kIU/L for CA 125 and CA 15-3.
could be fitted by a linear regression model, and the slope $b_1$ could be calculated. Except for TATI, the value of the slope $b_1$ was very small, which means that, although the evolution of tumor marker with time was linear, the concentration did not rise markedly during pregnancy.

CEA was slightly increased in four of nine samples from one woman (range, 3.2–3.5 mg/L). A linear evolution of CEA was found in one patient (slope $b_1$, 0.071). CA 125 concentration was higher than 40 kIU/L in four samples, three from one woman and one from a second one (5.3% of all samples; range, 49–63 kIU/L). A linear evolution of CA 125 was found in three patients (slope $b_1$, 0.36 ± 0.2).

CA 125 is the most studied tumor marker in pregnancy. In the first trimester of pregnancy, CA 125 was increased in very different percentages of cases according to several authors: 16% (11), 55% (2), 12.5% (3), and 60% (12). Taken during the course of the pregnancy, a high CA 125 was found in 11 of 46 pregnant women (12) (with one extreme value of 143 kIU/L) or in 8 of 100 pregnant women (3). Furthermore, increased CA 125 concentration was correlated with a higher pregnancy rate in women undergoing an in vitro fertilization program (13).

In our study, all CA15-3 values were within the reference interval (<30 kIU/L), although in five patients, a linear temporal evolution of this marker was seen: slope $b_1$, 0.21 ± 0.08. By a statistical analysis, Touitou and Bogdan (2) also showed increasing CA 15-3 but with concentrations in the usual range of values.

SCC concentrations increased over the cutoff value of 1.6 mg/L in eight samples coming from seven different women (10.5% of all samples; range, 1.7–4.3 mg/L). Except in one case, an increase occurred after 23 weeks of amenorrhea. Mean SCC concentration was higher after 30 weeks of amenorrhea than before 30 weeks. In two patients, a linear evolution of SCC was found with a slope $b_1 = 0.05 ± 0.01$. In previous studies, high concentrations of SCC were found in amniotic fluid until a mean concentration of 670 μg/L in the third trimester of pregnancy, but in maternal serum, SCC was within the reference interval (14).

In our study, TATI concentrations increased >25 μg/L in 13 samples from six different women, mainly in the third trimester of pregnancy (17.1% of samples; range, 27–65 μg/L). TATI can be particularly high in some patients, up to 65 μg/L, i.e., 2.6 times the cutoff value. Furthermore, we found a marked linear increase of TATI in four patients. The values for the slope $b_1$ were much higher than for the other markers: 1.15 ± 0.66. A slope higher than 1 (and a sharp increase in patient 8) was found in three women (1.23, 1.43, and 1.73; Fig. 1). TATI increased above the cutoff value of 25 μg/L relatively late but began to rise from the beginning of pregnancy, staying in the reference interval until 20 weeks of amenorrhea. To our knowledge, no other publication reports TATI concentrations in pregnancy.

CA 19-9 (a marker of gastrointestinal tract), tissue polypeptide antigen (TPA), and neurone-specific enolase (NSE; a marker of small cell lung carcinoma) have been studied in pregnancy. Increased CA 19-9 (>37 kIU/L)
was reported in 4.8% of 21 patients (2) and in 10% of 100 patients (range, 38–117 kIU/L) (3). In another study, serum CA 19-9 and NSE were not increased in 87 pregnant women (15).

TPA (a marker of cell proliferation) (1) was studied as a possible marker of pathological pregnancy as eclampsia. TPA was higher in hypertensive pregnant women than in nonhypertensive ones (16), and TPA concentration was correlated with the severity of the disease.

Our results thus show and confirm that during pregnancy, tumor markers are rarely higher than the cutoff value but can increase markedly, staying under the cutoff value as for CA 15-3 or rising above it as for TATI or SCC. Increases of TATI and SCC occurred more frequently and mostly in the last months of pregnancy.

References