Monitoring Cancer Antigen 125 in Serum of Ovarian Cancer Patients after Administration of $^{131}$I-Labeled F(ab')$_2$ Fragments of OC125 Antibody

Jochen Reinsberg, Birgit Schultes, Uwe Wagner, and Dieter Krebs

We evaluated the effect of repeated administration of OC125 F(ab')$_2$ fragments on cancer antigen (CA) 125 determination in 210 serum samples from 30 patients. We found falsely high CA 125 concentrations in 142 (68%) samples, using a homologous CA 125 enzyme immunoassay (EIA) with OC125 antibodies. The Truquant OV2 method, which involves two other murine antibodies, and the IMx CA 125 method, which uses sheep antibodies as capture antibodies, resulted in only slightly increased (false-positive) values in some samples with exceptionally high CA 125 EIA values. We measured falsely low CA 125 values in 37 (18%) samples with the Truquant OV2 method. Interferences could be eliminated by removal of serum IgG. Our results suggest that interferences are to some extent caused by anti-idiotypic IgG induced by OC125 administration. Assays involving nonmurine anti-CA 125 antibodies as capture antibodies seem to be most suited for CA 125 determination after OC125 treatment, but in every case an apparent increase of CA 125 after OC125 infusion should be validated.

**Indexing Terms:** anti-idiotypic antibodies - heterophile antibodies - radioimmunoassay - enzyme immunoassay

Since its development by Bast et al. (1), the monoclonal antibody OC125 has become an important clinical tool for the diagnosis and therapy of ovarian cancer (2-5). Immunoscintigraphy with $^{131}$I-labeled F(ab')$_2$ fragments of the OC125 antibody appears to be sensitive and specific for detecting metastases and recurrences of ovarian carcinoma (6, 7). In vivo administration of OC125 F(ab')$_2$ fragments is useful for therapy as well (8, 9). However, this procedure induces the formation of antibodies to the F(ab')$_2$. Some of these are anti-idiotypic antibodies directed against idiotopes within the hypervariable region of the OC125 F(ab')$_2$ fragments (10). Anti-idiotypic antibodies interfere with the in vivo radioimmunodetection of cancer antigen (CA) 125 by forming antigen--antibody complexes with the injected OC125 fragments. Such complexes accumulate in the reticuloendothelial system, leading to increased activity in liver and spleen (7, 11, 12).

Because the OC125 antibody recognizes a repeated epitope, it is common to measure CA 125 by means of the immunometric “two-side sandwich” technique, using the OC125 antibody as both the capture and the detector antibody (2). In such an assay, anti-idiotypic antibodies, which bind to an idiotope of OC125, can cross-link both antibodies, resulting in falsely high values for CA 125 (10, 13).

In one patient, no interference was seen with an immunometric assay that involved no OC125 antibodies (10). This prompted us to evaluate the effect of repeated administration of OC125 fragments on CA 125 determination by comparing the apparent CA 125 concentrations measured with the homologous enzyme immunoassay (EIA) of CA 125, involving only OC 125 antibodies, with the results of two other test kits, which involve at least one other antibody. To determine the real CA 125 concentration in discrepant samples, we established a procedure to remove interfering antibodies. We also investigated the relation between the concentration of nonspecific (iso/allotypic) human anti-murine antibodies (HAMAs) and the false-positive CA 125 concentration measured with the CA 125 EIA.

**Materials and Methods**

**Patients and serum samples.** All serum samples were obtained routinely during follow-up of ovarian cancer patients (Stage II–IV, according to the FIGO system). The samples were aliquoted and stored at -20 °C until analyzed.

Samples (n = 210) were drawn from 30 patients who had received one or more infusions of 1 mg of $^{131}$I-labeled F(ab')$_2$ fragments of the anti-CA 125 antibody OC125 (IMACIS-2; Isotopen Diagnostik CIS, Dreieich, Germany) for radioimmunodetection. Thirty-two samples, drawn from 21 of these patients before antibody treatment, served as controls. An additional 18 control samples were obtained from 12 other ovarian cancer patients not yet treated with OC125 antibodies.

**Determination of CA 125.** CA 125 was measured with the Abbott CA 125 EIA monoclonal and the IMx CA 125 (both from Abbott Diagnostic, Wiesbaden-Delkenheim, Germany) and the Truquant OV2 (Biomira Inc., Edmonton, Alberta, Canada) immunoradiometric assay. Each of the three tests is a solid-phase immunometric assay for CA 125. In the Abbott CA 125 EIA, the OC125 antibody is used as both the immobilized (capture) and the detector antibody. In the IMx CA 125, polyclonal sheep anti-CA 125 antibodies are used as capture antibodies; the OC125 antibodies serve only as enzyme-labeled detector antibodies. In the Truquant OV2 no OC125 antibodies are involved: CA 125 measurement is performed with two newly developed murine monoclonal antibodies (Truquant B43.13 and Truquant B27.1), which recognize separate epitopes near the sites recognized by OC125 antibodies (14). In the CA 125 EIA, binding of both antibodies takes place simultaneously during one incubation step. In the IMx CA 125 and the
Truquant OV2, the detector antibody is added only after all serum components not bound to the capture antibody are removed by washing. The three tests were performed according to the manufacturers’ instructions.

**Determination of human anti-murine antibodies.** HAMAs were determined with the ImmuSTRIP HAMA-EIA (Immunomedics Inc., Newark, NJ) as described before (10).

**Determination of human serum IgG.** Human serum IgG was determined by radial immunodiffusion on LC-Partigen IgG plates (Behring, Marburg, Germany).

**Removal of interfering IgG from serum samples.** Interfering IgG was removed by affinity chromatography on Protein G-Sepharose (Pharmacia, Freiburg, Germany). We applied 1 mL of serum samples diluted twofold with phosphate buffer (0.2 mol/L sodium phosphate and 10 g/L bovine serum albumin, pH 7.0) to the 2.5-mL column equilibrated with phosphate buffer. After a 5-min incubation at room temperature, the column was washed with 8 mL of phosphate buffer to elute the unadsorbed fraction. The eluted fraction was frozen immediately at −80 °C and subsequently lyophilized. Samples were reconstituted when needed with 1 mL of distilled water.

**Statistics and calculation.** Linear-regression analysis was performed by the Passing/Bablok method (15). As a measure of scatter of data points of the control collective, we calculated the geometric mean with the 99% tolerance interval for the ratio x/y, where x and y are the CA 125 concentrations measured with the respective test kits in one sample. Because the intercepts of the regression lines established for the control samples are not significantly different from zero, one can expect the ratio x/y for all samples to be scattered about a constant value representing the slope of the regression line. We chose this method because the scatter of data points described by the ratio x/y increased with increasing values of x, as expected for a comparison of methods with a nearly constant coefficient of variation rather than a constant measurement error at all values. In contrast, the standard error of the estimate normally used to detect outliers is constant at every value of x (16). A data point (xₐ, yₐ) was classified as deviating from the relationship of control samples when the ratio xₐ/yₐ exceeded the respective tolerance limits of the control collective.

**Results**

**Identification of samples containing interfering antibodies.** Serum samples obtained from patients after antibody treatment were screened for the presence of interfering antibodies by comparing the CA 125 concentrations measured with the CA 125 EIA, the Truquant OV2, and the IMx CA 125. Figures 1, 2, and 3 (upper panels) show the normal deviation of results of CA 125 determination with the three different tests for 50 control samples, together with the respective 99% tolerance intervals of the ratio x/y.

Figures 1, 2, and 3 (lower panels) show the results for 210 samples drawn after antibody treatment. For 68%
TRUQUANT OV2
(kilo-
arb.units/L)

TRUQUANT OV2
(kilo-
arb.units/L)

IMx CA 125 (kilo-ARB.units/L)

IMx CA 125 (kilo-ARB.units/L)

Fig. 3. Comparison of CA 125 concentrations measured with the Truquant OV2 and the IMx CA 125 in 50 control samples (top), and in 210 samples from patients treated with OC125 fragments (bottom) The dashed lines represent the limits of the 99% tolerance interval of the ratio x/y, calculated for the control collective.

Table 1. Recovery of Real CA 125 Concentration after IgG Removal in 10 Control Samples, Measured with the Three Assays

<table>
<thead>
<tr>
<th>Test</th>
<th>CA 125 conc. (kilo-ARB.units/L)</th>
<th>Recovery % a</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 125 EIA</td>
<td>102 – 2075</td>
<td>91 (80–105)</td>
</tr>
<tr>
<td>Truquant OV2</td>
<td>110 – 3454</td>
<td>99 (88–117)</td>
</tr>
<tr>
<td>IMx CA 125</td>
<td>65 – 1575</td>
<td>96 (87–113)</td>
</tr>
</tbody>
</table>

a Mean (range).

(142/210) of the samples, we found discrepantly high results with the CA 125 EIA compared with both the IMx CA 125 and the Truquant OV2 (Figures 1 and 2, lower panels). When comparing the results of the Truquant OV2 and the IMx CA 125, we found that with the Truquant OV2, 3% (6/210) of the samples yielded discrepantly high results and 18% (37/210) of the samples yielded discrepantly low results (Figure 3, bottom).

Removal of interfering antibodies. To determine more accurately the CA 125 concentrations of the discrepant samples, we attempted to remove putative interfering antibodies by means of affinity chromatography on Protein G–Sepharose. CA 125 concentrations in 10 control samples were not affected by chromatography (Table 1) when the IgG concentration was reduced to <30 mg/L.

Table 2 shows the effect of IgG removal on the

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Pre</th>
<th>Post</th>
<th>Recovery, %</th>
<th>Pre</th>
<th>Post</th>
<th>Recovery, %</th>
<th>Pre</th>
<th>Post</th>
<th>Recovery, %</th>
<th>Pre</th>
<th>Post</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>377 000</td>
<td>1 900</td>
<td>&lt;1</td>
<td>119</td>
<td>&lt;20</td>
<td>&lt;17</td>
<td>36</td>
<td>&lt;20</td>
<td>&lt;56</td>
<td>20</td>
<td>&lt;20</td>
<td>&lt;3</td>
</tr>
<tr>
<td>2</td>
<td>421 000</td>
<td>2 600</td>
<td>&lt;1</td>
<td>188</td>
<td>&lt;20</td>
<td>&lt;10</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 772</td>
<td>&lt;20</td>
<td>&lt;11</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;3</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2 080</td>
<td>&lt;20</td>
<td>&lt;1</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;3</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2 100</td>
<td>&lt;20</td>
<td>&lt;1</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;3</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>14 700</td>
<td>280</td>
<td>2</td>
<td>23</td>
<td>25</td>
<td>109</td>
<td>73</td>
<td>80</td>
<td>110</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2 180</td>
<td>60</td>
<td>3</td>
<td>139</td>
<td>175</td>
<td>128</td>
<td>113</td>
<td>111</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4 880</td>
<td>135</td>
<td>3</td>
<td>234</td>
<td>224</td>
<td>96</td>
<td>188</td>
<td>178</td>
<td>94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>494</td>
<td>89</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td>534</td>
<td>590</td>
<td>110</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2 100</td>
<td>179</td>
<td>9</td>
<td>420</td>
<td>419</td>
<td>100</td>
<td>684</td>
<td>647</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>35 800</td>
<td>960</td>
<td>2</td>
<td>990</td>
<td>1 050</td>
<td>106</td>
<td>47</td>
<td>44</td>
<td>94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3 480</td>
<td>790</td>
<td>23</td>
<td>1 600</td>
<td>1 700</td>
<td>106</td>
<td>347</td>
<td>404</td>
<td>116</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>481</td>
<td>100</td>
<td>21</td>
<td>52</td>
<td>100</td>
<td>192</td>
<td>454</td>
<td>383</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>5 700</td>
<td>430</td>
<td>8</td>
<td>574</td>
<td>907</td>
<td>158</td>
<td>171</td>
<td>157</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>12 300</td>
<td>1 200</td>
<td>10</td>
<td>453</td>
<td>932</td>
<td>206</td>
<td>258</td>
<td>235</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>2 750</td>
<td>190</td>
<td>7</td>
<td>86</td>
<td>271</td>
<td>315</td>
<td>704</td>
<td>720</td>
<td>102</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>4 340</td>
<td>271</td>
<td>6</td>
<td>140</td>
<td>675</td>
<td>482</td>
<td>1 180</td>
<td>970</td>
<td>82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 a</td>
<td>477 000</td>
<td>3 700</td>
<td>&lt;1</td>
<td>355</td>
<td>978</td>
<td>278</td>
<td>980</td>
<td>980</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>51 400</td>
<td>1 300</td>
<td>3</td>
<td>1 030</td>
<td>2 100</td>
<td>204</td>
<td>9 000</td>
<td>8 960</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 b</td>
<td>45 500</td>
<td>790</td>
<td>2</td>
<td>592</td>
<td>2 340</td>
<td>395</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>63 500</td>
<td>13 000</td>
<td>20</td>
<td>828 15 200</td>
<td>1 836</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Recovery not calculated.
b,c Obtained from one patient.
apparent CA 125 concentrations for 21 samples drawn from 19 patients after antibody treatment. After IgG removal, the apparent CA 125 concentrations measured with the CA 125 EIA were markedly reduced in all samples (mean recovery <7%). As shown in Figure 4 (top and middle panels), the reduced concentrations corresponded well with the results of the Truquant OV2 and the IMx CA 125. However, for four samples (no. 1, 2, 6, and 18) the reduced concentrations still considerably exceeded the values expected according to the control collective.

In contrast, the apparent CA 125 concentrations measured with the Truquant OV2 were almost unchanged after IgG removal in 10 samples (nos. 3–12). Only in the first 2 samples with discrepantly high results before chromatography were the concentrations reduced. However, in samples 13–21 we found distinctly higher results after chromatography (mean recovery 452%). In the last six of these samples, before chromatography, we had found discrepantly low results. As shown in Figure 4 (bottom), all discrepant results found with the Truquant OV2 compared with the IMx CA 125 were corrected by IgG removal.

The CA 125 concentrations measured with the IMx CA 125 were recovered almost completely after IgG removal in 20 samples. Only in the first sample was apparent CA 125 slightly reduced.

**Dynamics of interfering antibodies.** To confirm that the interfering antibodies are induced by administration of OC125 fragments, we analyzed the relation between the time of antibody infusion and the appearance of discrepant CA 125 results. Figure 5 (top) shows a typical time course of apparent CA 125 concentrations for a patient with constant low real CA 125 who received two antibody infusions. Three weeks after the first infusion, the apparent CA 125 concentration measured with the CA 125 EIA dramatically increased, whereas the values measured with the Truquant OV2 and the IMx CA 125 did not exceed 20 kilo-arbitrary (arb.) units per liter. After the second infusion, an additional increase up to values >10⁶ kilo-arb. units/L was measured with the CA 125 EIA. Simultaneously, with the Truquant OV2 and to a lesser extent with the

---

**Fig. 4. Comparisons of apparent CA 125 concentrations measured with the CA 125 EIA, the Truquant OV2 and the IMx CA 125 in 21 discrepant samples before (□) and after (○) IgG removal: (top) CA 125 EIA compared with Truquant OV2, (middle) CA 125 EIA compared with IMx CA 125, (bottom) Truquant OV2 compared with IMx CA 125.**

**Fig. 5. Typical time course of apparent CA 125 concentration measured with the CA 125 EIA (■), the Truquant OV2 (●), and the IMx CA 125 (□) during repeated treatment with OC125 fragments in a patient with constant low real CA 125 concentration (top), and in a patient with increasing real CA 125 concentration (bottom).**

Arrows indicate the time of OC125 infusions.
IMx CA 125, a slight apparent increase of CA 125 was measured.

Figure 5 (bottom) shows the time course for a patient with increasing real CA 125 due to a progressing tumor, who received the second antibody infusion. The apparent CA 125 concentration measured with the CA 125 EIA was already falsely high because of the preceding infusion, and increased further after the second infusion. The real CA 125, in this case determined correctly by the IMx CA 125, increased continuously from 130 to 980 kilo-arb. units/L, corresponding with the clinical course of disease. In contrast, with the Truquant OV2 we initially measured an increase of the CA 125 concentration, but after antibody infusion, the apparent CA 125 concentration decreased.

**Human anti-murine antibodies.** To clarify how much interference was due to increased concentrations of nonspecific (anti-iso/allotypic) HAMAs, we examined the relation between HAMa concentrations and falsely high CA 125 concentrations measured with the CA 125 EIA in samples with real CA 125 concentrations <20 kilo-arb. units/L. In these samples the results almost exclusively reflected the effect of interfering antibodies. Correlation between HAMa concentrations and falsely high CA 125 concentrations (Figure 6) was good (r = 0.885, n = 61). However, there were outliers for samples with increased HAMa concentrations up to 1.3 mg/L but with low results in the CA 125 EIA.

**Discussion**

In several serum samples drawn after infusion of OC125 fragments, interfering agents led to false results for CA 125 determination. We demonstrated four types of discrepancies: falsely high results in the CA 125 EIA (high incidence), falsely high results in the Truquant OV2 (low incidence), falsely high results in the IMx CA 125 (low incidence), and falsely low results in the Truquant OV2 with a reduction of assay response up to 90% (probable high incidence). The fact that the interfering agents could be absorbed by Protein G, and the relation between the time of infusions and the appearance of interference, strongly suggested that the interfering agents were human IgGs induced and boosted by injection of OC125 antibodies.

Falsely increased values in most samples were observed only with the CA 125 EIA. This supports the hypothesis that the responsible IgGs are anti-idiotypic antibodies highly specific to OC125 antibodies, with no affinity to the other anti-CA 125 antibodies involved in the Truquant OV2 and the IMx CA 125. HAMAs did not interfere with the CA 125 EIA, at least up to a concentration of 1.3 mg/L, presumably because the CA 125 EIA, as well as the Truquant OV2 and the IMx CA 125, included nonspecific murine IgGs to block the activity of HAMAs (17, 19). In most of the discrepant samples the HAMA concentrations did not exceed this limit. This agrees with previous results in which we demonstrated for one patient the existence of anti-idiotypic antibodies after treatment with OC125 fragments, producing falsely high results in the EIA but not in the Truquant OV2 (10). Falsely high results in CA 125 determination due to antibodies induced by treatment with OC125 fragments also have been reported by Muto et al. (20).

The interfering antibodies were found to be capable of binding OC125 antibodies but the further specificity of the antibodies was not examined.

In a few samples with exceptionally high false-positive CA 125 EIA values accompanied by exceptionally high HAMA concentrations, we observed falsely high results with the Truquant OV2 and to a lesser degree with the IMx CA 125. This effect becomes evident only in samples with low real CA 125 concentrations. As demonstrated by Boscato and Stuart (19), in samples with very high HAMA concentrations the amount of nonspecific murine immunoglobulins added to an assay system may not always be enough to block interferences by HAMAs. Falsely high results in the Truquant OV2 and the IMx CA 125 may be due to HAMAs not further blocked by nonspecific murine immunoglobulins.

In four samples with very high false-positive results in the CA 125 EIA we could not eliminate interferences completely by removing serum IgG by affinity chromatography on Protein G-Sepharose. In those samples the trace of IgG remaining after chromatography was enough to show considerably increased CA 125 concentrations.

The most prominent interference observed with the Truquant OV2 was the reduction of assay response, resulting in falsely low CA 125 values. This can be caused by high HAMA concentrations, presumably because of inhibition of antigen binding to reagent antibodies (21, 22). However, we cannot exclude the possibility that anti-idiotypic antibodies induced by OC125 injection bind to the antigen-binding site of the capture antibodies used in the Truquant OV2 but not to the detector antibodies. In this case, anti-idiotypic antibodies cannot produce falsely positive results but they can displace and inhibit binding of CA 125. The fact that this inhibition does not occur with the IMx CA 125 suggests that the inhibiting antibodies have no affinity to the sheep antibodies used as capture antibodies in the IMx CA 125. Although the use of antibodies from
different animals does not always eliminate interference due to HAMAs (21, 23), in this case it may.

CA 125 concentrations in patients treated with OC125 fragments should be interpreted with care. In such patients determination of CA 125 should not be performed by means of a homologous assay involving only OC125 antibodies, neither in the native sample nor after IgG removal, because of the very high incidence of false-positive results. The use of test kits involving other antibodies cannot completely eliminate interferences in the native samples. Of the test kits studied, the IMx CA 125 seems to be the most suited for monitoring CA 125 in samples from patients treated with OC125 fragments. Nevertheless, even with this assay, increased CA 125 values seen after OC125 administration should be validated to rule out a false-positive increase caused by interference from newly formed antibodies.

References

Corrections

Vol. 36

p. 517. In the article by L.H. Bernstein, R.A. Rudolph, M.M. Pinto, N. Viner, and H. Zuckerman entitled "Medically significant concentrations of prostate-specific antigen in serum assessed," 1990;36:515–8, the text describing Table 4 reports reversed values for sensitivity and positive predictive value and likewise for specificity and negative predictive value. The text should read, "Table 4, a reorganized Table 3, defines the occurrence of binary class patterns with respect to carcinoma of the prostate by stage compared with nondisease. The table allows the estimation of 44.4% sensitivity, 97.1% specificity, a negative predictive value of 79%, and a positive predictive value of 87.8% for PSA >22.8 ng/mL."

Vol. 36

p. 2557. In the letter to the editor by J.D. Mitchell, B.J. Perrigo, and V.A. Mason-Daniel entitled "Falsey negative urine drug assay results due to filtration," 1992;38:2556–7, the units in the first paragraph at the top of p. 2557 should have been micrograms per liter (µg/L), not pg/L.