An Enzyme Immunoassay Procedure for Cancer Antigen 125 Evaluated

Kenneth W. Ryder, Tjlen O. Oel, Meredith T. Hull, and Martha M. Sample

The performance of a new enzyme immunoassay (EIA) procedure (Abbott Labs.) for cancer antigen 125 (CA 125) met or exceeded the manufacturer’s claims for all analytical variables examined. Overall correlation with results obtained with a radioimmunoassay (RIA) were good. However, near the decision thresholds typically chosen to define a positive result for ovarian carcinoma, EIA results were 10 to 20 arbitrary units/mL less than the RIA results. At specific decision thresholds, therefore, the sensitivities and specificities of the EIA and RIA procedure differed. Adjusting the decision thresholds gave a similar optimum efficiency for each procedure: EIA, 82.9% (decision threshold, 35 units/mL); RIA, 83.4% (decision threshold, 54 units/mL). Receiver–operating characteristic curves showed that the two procedures’ ability to distinguish patients with active ovarian carcinoma from those with disease in remission was the same.

The poor prognosis of patients with ovarian carcinoma results in part from the absence of specific early symptoms. This has stimulated the search for a reliable marker of this disease in serum (1). A monoclonal antibody (OC 125) has been developed that reacts with a surface marker, cancer antigen 125 (CA 125), which is expressed on more than 80% of ovarian carcinomas (2, 3). In serum, CA 125 is associated with a high-molecular-mass glycoprotein. With a radioimmunoassay of CA 125, a positive test result (defined as CA 125 >55 arbitrary units/mL) was found in 82% of patients with ovarian carcinomas, 6% of patients with nonmalignant diseases, and 1% of healthy individuals (4, 5).

In general, changes in the concentrations of CA 125 in serum correlate well with disease activity (6–14). Therefore, a positive CA 125 test result for the patient who has completed initial surgery and a first course of chemotherapy may indicate the persistence of disease, obviating the usual "second-look" laparotomy to assess disease status (15). A negative CA 125 result under such circumstances, however, does not always correlate with lack of disease, particularly disease that is only microscopically detectable (8, 10, 12, 14).

CA 125 in serum has been measured by radioimmunoassay (2, 16). Here we report our evaluation of a new enzyme immunoassay (EIA) procedure recently developed for CA 125.

Materials and Methods

We obtained 198 serum samples from 82 patients with ovarian neoplasms. Of these, 95 were from patients with active disease and 103 from patients with disease in remission. In all cases, disease status, assessed on the date the sample was obtained, was based on histological assessment of biopsies for patients with active disease. For patients with inactive disease, this assessment was based on lack of disease as evaluated by histological evaluation of biopsy, radiographic studies including computerized axial tomography, and (or) clinical assessment.

CA 125 was doubly measured in each sample: by radioimmunoassay (performed by SmithKline Bio-Science Laboratory, St. Louis, MO 63146) and by the EIA method we describe here (Abbott Laboratories, North Chicago, IL...
60064), a simultaneous solid-phase "sandwich"-type immunoassay (17). In the EIA, beads coated with monoclonal anti-CA 125 are incubated with the specimen and with monoclonal anti-CA 125 conjugated to horseradish peroxidase (EC 1.11.1.7). After incubation, unbound materials are removed by washing and the beads are incubated with α-phenylenediamine substrate containing hydrogen peroxide. The intensity of color formed is proportional to the amount of CA 125 in the sample and is measured spectrophotometrically at 492 nm. Results are expressed in arbitrary units.

Studies of correlations between EIA and RIA results included the following:
- Diagnostic sensitivity, specificity, and efficiency of each procedure (18),
- Split-sample comparisons of all results and of the subset of results near the decision threshold (19), and
- Receiver–operating characteristic curves for each test as described by Beck and Schultz (20).

We also assessed the analytical performance of the EIA procedure by evaluating:
- The effect of potential interference from lipemia, bilirubin, and hemolysate, assessed by using interferographs as previously described (21). The maximum concentrations studied were: hemolysate (as hemoglobin), 10 g/L; bilirubin, 0.6 g/L; lipemia (added Intralipid), 10 g/L.
- The reference interval, evaluated from data on serum samples from 22 normal, nonpregnant women volunteers.
- The detection limit, as determined from 20 replicate analyses of the zero standard (supplied by the manufacturer) by calculating their mean + 2 SD (17).

Results and Discussion

The distribution, as assessed by histology, of patients with ovarian neoplasms is shown in Table 1. Of the 82 subjects in this study, 71 (87%) had ovarian carcinomas. Figure 1 shows the distribution of CA 125 results for patients with active disease and patients with inactive disease, as measured by the EIA procedure.

To compare CA 125 results obtained by EIA and RIA, we performed a split-sample correlation study. Correlation and range of results were as follows: range (RIA), <7 to 17 549 units/mL; range (EIA), <5 to 18 800 units/mL; mean (RIA), 455 units/mL; mean (EIA), 461 units/mL; slope (m), 1.03; intercept (b), −7 units/mL; correlation coefficient (r), 0.98; S_{xy}, 349 units/mL.

Although this overall correlation analysis is favorable, we noted that, near the decision thresholds typically used to define a positive CA 125 test, most EIA results were 10 to 20 units/mL less than the corresponding RIA results. Correlation of the 159 samples with CA 125 <100 units/mL gave the following: mean (RIA), 34.6 units/mL; mean (EIA), 19.5 units/mL; m, 0.77; b, −7 units/mL; r, 0.84; S_{xy}, 15.4 units/mL. For all but three of 142 specimens (96%) the EIA value was less than the RIA value. All of these three specimens were from a single patient.

Because of this 15.1 unit/mL difference in means (EIA < RIA), we examined the difference in sensitivity and specificity of these procedures at the decision threshold most commonly used to define a positive test (35 units/mL) for patients with active (Table 2) and inactive (Table 3) disease. At this decision threshold the sensitivity of the EIA procedure (75%) is less than the sensitivity either observed here (83%) or reported in other studies (81–85%) (4, 9, 22) for the RIA procedure. As expected, however, the better sensitivity of the RIA procedure was offset by lower specificity as compared with the EIA method (RIA, 82%; EIA, 90%) procedure. The optimal efficiency for the RIA procedure was 83.4% if we used a decision threshold of 54 units/mL. The optimal efficiency for the EIA procedure, 82.9%, was obtained with a decision threshold of 35 units/mL. At these decision thresholds (Tables 2 and 3) the sensitivities of the procedures are similar (EIA, 75%; RIA, 74%) and the specificities of the procedures are more nearly the same: EIA, 90%; RIA, 93%. Results for the subgroup of patients with ovarian carcinomas were nearly identical.

Because the efficiency of the two methods was almost identical when the optimal decision threshold was used, we prepared ROC curves (Figure 2) to see if either procedure better distinguished active disease from disease in remission. Although the sensitivity and specificity varied, as expected, at particular thresholds, the areas under the two curves were identical, indicating that both tests have equal discriminating power for the presence of active disease vs disease in remission (20).

We also examined the analytical performance of the EIA procedure. We confirmed the manufacturer’s claim that

<table>
<thead>
<tr>
<th>Histological assignment</th>
<th>Active disease</th>
<th>Remission</th>
</tr>
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<tbody>
<tr>
<td>Ovarian carcinomas</td>
<td>43 (91)*</td>
<td>28 (80)</td>
</tr>
<tr>
<td>Serous</td>
<td>26 (55)</td>
<td>68 (72)</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>15 (32)</td>
<td>21 (22)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>1 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Epithelial ovarian tumors</td>
<td>2 (4)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Borderline, serous</td>
<td>1 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Borderline, mucinous</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Benign, serous</td>
<td>1 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Benign, mucinous</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Germ-cell/sex-cord tumors</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stromal tumors</td>
<td>2 (4)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>47(100)</td>
<td>95(100)</td>
</tr>
</tbody>
</table>

*Number (and percent) of specimens in each group.
dilution and re-assay are not required if the CA 125 result is <650 units/mL. Only 16 of 198 (8%) of our specimens required dilution and re-assay. The manufacturer states that the detection limit of the EIA procedure is about 5 units/mL; however, we calculated a detection limit of 0.2 units/mL, indicating that the detection limit is better than that stated by the manufacturer.

The reference interval for CA 125 in the healthy population measured with the RIA procedure reportedly is 20–40 units/mL (12, 23), although values >300 units/mL have been observed (24). We used the EIA procedure to measure CA 125 in 22 healthy nonpregnant female volunteers. The range of results we observed in this group (0–6 units/mL) was lower than noted in the reports where an RIA procedure had been used.

We assessed the effect of the potential interfering substances lipemia, bilirubin, and hemoglobin, by use of interferographs. We found no effect on this procedure at the concentrations used in this study.

We found the analytical performance of the EIA test for CA 125 to be excellent, and we believe that it can be used as an alternative to the RIA procedure. No overall difference between the EIA or RIA procedures for CA 125 was noted when these were used to discriminate patients with active disease from those with disease in remission.

The bias of 15 units/mL (EIA < RIA) produced important differences in the sensitivity and specificity of each test at particular decision thresholds. These differences affect the definition of a positive CA 125 test result in deciding which patients would benefit from second-look laparotomy. In such cases a different decision threshold should be used if the EIA procedure is substituted for the RIA method.

Abbott Laboratories provided kits and financial support for this study.

References
17. Package insert, Abbott CA 125-EIA Monoclonal. North Chica

Materials and Methods

Urinary albumin was measured by enzyme-linked immunoassorbent assay as described previously (9). Urine samples were collected in 2-L casein-coated polyethylene containers (9). Albumin excretion was measured in 24-h collections of urine from 66 apparently healthy subjects (eight men and 10 women, ages 20-29 y; 10 men and 10 women, ages 30-39 y; eight men and 10 women, ages 40-49 y; and five men and five women, ages 50-55 y). In 30 of those subjects (five of each sex in the age ranges 20-29, 30-39, and 40-50 y) the 24-h collections were made as two 12-h collections (one from 0700 to 1900 hours, the second overnight from 1900 to 0700 hours) to contrast night-time with daytime excretion. With the same 30 subjects, we estimated albumin excretion rate before (period I), during and shortly after (period II), and after (period III) strenuous exercise. Period I lasted from 0700 to the start of the exercise, period II from the start of the exercise until 1 h after the end of the exercise, and period III from 1 h after the exercise until 0700 hours on the next day.

Results from periods I, II, and III were pooled to provide a 24-h period including exercise, which could then be compared with a 24-h period without exercise in the same subject. The two 24-h urine collections were done within one week, but not on consecutive days. The subjects performed strenuous exercise on a bicycle ergometer (Reiper Dynavit; Meditronic 40/2, 6750 Kaiserslautern, F.R.G.), according to the following protocol: Start with a workload of 30 W (J/s, or kg·m²·s⁻³) for 3 min and increase to 70 W for 3 min, then further increase the workload by 40 W every 3 min until physical exhaustion. Blood pressure was measured with a sphygmomanometer. A statistical evaluation was by means of paired t-test; data are reported as mean ± SEM.

Influence of Strenuous Exercise on Albumin Excretion

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Renal albumin excretion rate was 7.3 mg/24 h (SEM 0.5, range 0.6-21.0) in 66 healthy subjects. This rate increased markedly during and shortly after strenuous exercise on a bicycle ergometer (before: 5.5 ± 0.6 μg/min; during and just after: 16.9 ± 2.2 μg/min; P <0.001; n = 30). However, albumin excretion/24 h was not significantly higher during 24 h with a period of strenuous exercise than during 24 h without such exercise (10.3 ± 0.9 mg/24 h vs 8.5 ± 0.7 mg/24 h).

Additional Keyphrases: albuminuria · diabetes · diabetic nephropathy

End-stage renal disease develops in about 40% of insulin-dependent diabetics (1, 2), overt proteinuria (>500 mg of protein or >300 mg of albumin excreted in 24 h) being the hallmark of diabetic nephropathy and a serious prognostic marker (1-3). Pauci("micro")albuminuria is a strong predictor of future overt nephropathy (4, 5) in diabetic patients. Early paucialbuminuria can be stopped or even reversed by means of better control of blood glucose or of blood pressure (3, 6, 7), thus probably slowing or even preventing development of overt diabetic nephropathy. Exact estimation of a slight albuminuria is also of interest for the control of patients with hypertension (8). Our aim in this study was to examine whether 24-h albumin excretion is altered misleadingly by a rather brief period of strenuous exercise during the urine-collection period.

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