activity should be expressed per 10¹² RBC or per gram of Hb (11). The excellent correlation we found between the two implies that, at least in the patient group we studied, either method can be utilized with equal accuracy.

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References

CA 125 Determined by Three Methods in Samples from Patients with Human Anti-Mouse Antibodies (HAMA), Ursula Turpeinen,1,2 Pentti Lehtovirta,2 and Ulf-Håkan Stenman1 (Depts. of 1 Clin. Chem. and 2 Obstet. and Gynecol. at Helsinki Univ. Central Hosp., Haartmaninkatu 2, 00290 Helsinki, Finland; author for correspondence: fax +358-0-471-4084)

Infusion of OC125 fragments in connection with radio-imaging of ovarian tumors often causes formation of anti-mouse IgG antibodies (HAMA), which can lead to falsely high results in OC125-based homologous immunoassays for CA 125 (1-5). The interference can be reduced by addition of mouse IgG. However, the antibodies are often anti-idiotypic, i.e., directed against idiotypes within the hypervariable region of OC125, such that interference by these is not blocked by mouse IgG (1, 2). We recently described a chromatographic method for separating immunoglobulins from CA 125 before assay (2). However, the method is time consuming and dilutes the samples, which reduces the accuracy of the assay.

The CA 125 assay is especially prone to HAMA effects because the same monoclonal antibody (MAb) is used both on the solid phase and as a tracer and because the idiotype of OC125 appears to be highly immunogenic. In the first-generation Byk-IRMA (IRMA-mat*, Byk-Sangtec Diagnostica, Dietzenbach, Germany), anti-idiotypic antibodies, which bind to an idiotype on OC125, can cross-link solid-phase and detector antibody, resulting in falsely high values for CA 125 (1-3).

It should be possible to reduce the interference by anti-idiotypic antibodies by developing assays based on antibodies other than that used for injection. The recently introduced Truquant® OV2™ (Biomira, Edmonton, Canada) is a two-step heterologous double-determinant solid-phase assay that utilizes the B27.1 mouse MAb as a capture antibody to bind molecules containing OC125-reactive determinants (6). Quantification is obtained by using B43.13 as a detector antibody, and assay calibration is based on a reference preparation maintained by Biomira. The Centocor CA 125 II assay (Centocor, Malvern, PA) is a one-step assay using the M11 antibody as a capture antibody and the OC125 antibody as a detector antibody (7). Assay calibration is based on a Centocorn maintained reference preparation. In our two-step modification of the assay, the sample is first incubated overnight with the capture antibody and, after washing, incubated for 2 h with the second antibody. To reduce the HAMA interference in the modified Centocor CA 125 II assay, we preincubated serum samples for 2 h at room temperature with 20 µL of mouse serum (Dakopatts, Glostrup, Denmark) per 100 µL of serum.

Because the second-generation CA 125 assays have replaced the original CA 125 tests, it is important to assess the effect of HAMA on these new assays. To determine the real CA 125 concentration in discrepant samples, we used cation-exchange chromatography to remove interfering antibodies (2). HAMA was determined by an immunofluorometric method (2) and by the Truquant® HAMA-RIA (Biomira). Immunoscintigraphy was performed with a 131I-labeled F(ab')2-fragment of the OC125 antibody Imacis-2 (ORIS, Gif-sur-Yvette, France) (8).

Figure 1A shows a comparison between the Truquant OV2 (γ) and Byk-IRMA (x) CA 125 methods for 35 control samples and 29 samples drawn 0.5–2 years after immunoscintigraphy. For the control samples, the correlation between the methods is good: r = 1.0, y = 0.95 x + 1.52. However, in 72% of the samples obtained after immunoscintigraphy, results were clearly higher with the Byk-IRMA than with the Truquant OV2. This suggests that bridging of the two MAbs by HAMA was responsible for the increase in CA 125 concentrations in the Byk-IRMA.

To determine the true CA 125 concentration of the discrepant samples, we removed HAMA by cation-exchange chromatography (2). Fig. 1B shows the effect of HAMA removal on the apparent CA 125 concentrations measured by the Byk-IRMA and by the Centocor one-step assay (Fig. 1C) in samples from five patients obtained after immunoscintigraphy. After HAMA removal, the apparent CA 125 concentrations were markedly reduced in practically all samples. When the Centocor assay was performed in two steps and mouse serum was added to the samples before assay, the interference of HAMA seen in the one-step assay was strongly reduced and apparently completely eliminated. The concentrations obtained with the two-step modification were in the most cases similar to those obtained by Byk-IRMA after HAMA removal. How-
125 concentrations measured with the Byk-IRMA was fairly good ($r = 0.78$), indicating that in these samples the CA 125 results mainly reflected the effect of interfering antibodies.

To ascertain whether HAMAs were completely removed by cation-exchange chromatography, we analyzed six HAMA-containing samples from a patient before and after chromatography by measuring the concentration of HAMA by two different methods. Both HAMA assays indicated that the interfering antibodies had been completely removed. In three of six samples, the Truquant OV2 assay gave falsely low results when HAMA were present. When HAMA were removed, the Truquant OV2 assay values increased but still remained lower than those obtained by the Byk-IRMA.

In the Truquant OV2 assay, most samples with a high HAMA content gave falsely low CA 125 values, as also reported in a recent study (5). This suggests that antibodies bind to the idiotype of the capture antibody, thereby displacing CA 125 from the solid-phase antibody. However, the anti-idiotype antibody does not bind to the tracer antibody, and a falsely low result is obtained. Apparently, the idiotype of the B27.1 solid-phase antibody is very similar to that of OC125 but different from that of the B43.13 tracer antibody, even though both antibodies are thought to react with epitopes near but not identical to that binding OC125 (6).

With HAMA-containing samples, the Centocor CA 125 II assay showed a false-positive effect, which suggests that antibodies other than anti-idiotypic ones caused cross-linking of solid-phase and tracer antibody. This interpretation is supported by the finding that the effect could be eliminated by adding mouse serum and performing the assay in two steps. This procedure effectively reduced the binding of HAMA, and the anti-idiotypic antibodies to OC125 were removed by washing before the OC125 tracer antibody was added. This approach appears to be the most simple way of measuring CA 125 in samples containing antibodies to OC125. It may even be more reliable than chromatographic removal of HAMA, because after removal our results with the Byk-IRMA were still higher and those with the Truquant were lower than with the modified CA 125 II assay. Although we could not detect HAMA after chromatographic treatment, the HAMA assays used probably do not detect specific anti-idiotypic antibodies.

The amount of mouse serum we used in this study may not be sufficient to inhibit HAMA in all samples. This needs to be estimated by adding increasing amounts of mouse serum IgG to the sample before assay. In recent studies, the CA 125 II assay with HAMA-containing samples has yielded falsely low or normal results (9, 10), probably because of differences in the relative concentration of anti-idiotypic and immunoglobulin-specific HAMA in individual samples, respectively.

Interestingly, the HAMA causing a false increase of CA 125 in the Centocor CA 125 II assay did not cause a false increase in the Truquant assay. Apparently, the HAMA induced by OC125 do not effectively cross-link the two antibodies used in the Truquant assay, perhaps because of large differences in antigenic properties between the antibodies used and OC125 or the use of high concentrations of mouse IgG in the assay buffer. The use of two incubation steps with washing in between may also help reduce the cross-linking effect. A negative interference has also
been observed in another study (9). Although a negative net effect of OC125-induced HAMA may be typical in the Truquant assay, we cannot exclude the possibility that a cross-linking effect may dominate in samples from some patients.

Removal of OC125-induced HAMA can be achieved with affinity chromatography on Protein G–Sepharose (5) or MonoS ion-exchange chromatography (2). However, some HAMA may remain after Protein G chromatography (5), and this appears to be the case with MonoS in some samples we studied. The simplest and most reliable solution appears to be our modification of the new CA 125 II assay. On theoretical grounds, this assay is likely to give the most accurate results, and this possibility was supported by longitudinal studies showing no HAMA-induced changes in CA 125 concentrations. Furthermore, the results were compatible with the clinical course of the disease. Although this method seemed to work well with the samples studied, it must be evaluated with a larger number of samples before it can be recommended for general use.

References