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False Positive Results in an Enzyme Immunometric Assay for the Ovarian Cancer Associated Antigen CA 125

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Abstract—*A commercially available two-site, solid phase enzyme immunoassay was used for measuring CA* 125. *Samples from six healthy subjects with known false positive CA* 125 *results (range* 42–895 U/ml) *were retested after addition of murine serum. A decrease ranging from* 45 *to* 81% *in the CA* 125 *levels was seen after addition of* 63 ml/l *murine serum. After addition of* 500 ml/l *three out of six samples had antigen levels below* 35 U/ml. *It is concluded that in the present study false positive CA* 125 *results could be diminished by addition of murine serum to the sample and may be due to human antibodies against murine immunoglobulins. Addition of murine serum to samples from persons with no evidence of disease and increased CA* 125 *levels is recommended.*

INTRODUCTION

THE TUMOUR MARKER, cancer antigen 125 (CA 125), appears to be of importance in the monitoring of epithelial ovarian cancer. Small amounts of CA 125 are detectable in serum from healthy women and in most studies a content of 35 U/ml is considered as the upper limit of normal values [1]. However, CA 125 levels above 35 U/ml have been measured in females free of disease [1] and no explanation for these false positive results has been given, so far.

False positive results due to non-analytic interference in two-site immunometric assays for proteins and peptides have been described [2–4] and because of their high incidence they were found to be of importance using the assays for diagnostic purposes. The false positive results could be detected by adding serum from the animal species in which the applied immunoglobulins had been raised.

In the present study the effect of adding murine serum to samples with a false high CA 125 level is described.

MATERIALS AND METHODS

A commercially available two-site solid phase enzyme immunoassay (Abbott CA 125-EIA monoclonal) was used for the analyses of CA 125. One hundred microlitres of the sample were mixed with 100 μl of peroxidase conjugated, murine, monoclonal anti-CA 125 (OC125) and incubated for 4 h at 37°C with an OC125 coated bead. The beads were washed (QwikWash™) in demineralized water, and 300 μl of *o*-phenylenediamine was added to each sample followed by incubation for 30 min. The colour reaction was stopped adding 1 ml of 0.5 M sulphuric acid to each sample and the colour was measured (Quantum™ II, dual wave length analyser, 492:600 nm). The antigen concentration was read from a point to point standard curve using four standards (0, 65, 325 and 650 U/ml). All measurements were performed in duplicate.

CA 125 was measured in five serum and one plasma samples from healthy subjects with known false positive CA 125 results. Serum samples from three patients with ovarian cancer served as controls. CA 125 was measured before and after the addition of murine serum which was obtained from healthy mice.

All samples were stored at −80°C until analysis.

RESULTS

Murine serum was added to five serum and one plasma samples (42–895 U/ml) with false positive CA 125 results (Table 1). In all samples a marked decrease (range 45–81%) in the CA 125 values was observed after addition of 63 ml/l murine serum and in two of the samples the antigen level reached

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Table 1. *CA* 125 *before and after addition of murine serum*

	CA 125 (U/ml)					
	Without murine serum	With murine serum				
		63 ml/l	125 ml/l	250 ml/l	500 ml/l	900 ml/l
Serum 1	895	493	286	141	63	<5
Serum 2*	199	104	116	121	116	
Serum 3*	107	46	49	53	31	
Serum 4*	75	14	10	21	14	
Serum 5*	42	16	<5	<5	10	
Plasma 1*	507	268	248	246	190	
Patient 1	406				425	
Patient 2	277				240	
Patient 3	10				8	

*Supplied by Abbott Laboratories, Chicago, U.S.A.

normal (≤35 U/ml). Increasing amounts (125 and 250 ml/l) of murine serum were added but the CA 125 values were only slightly affected with the exception of serum 1 (895 U/ml). At the addition of 500 ml/l the antigen level was normalized in one additional sample. The CA 125 level in the sample with the highest antigen value (895 U/ml) gradually decreased after addition of 125, 250 and 500 ml murine serum per litre and at the addition of 900 ml/l murine serum the antigen could no longer be traced.

Serum samples from three patients with ovarian carcinomas (range 10–406 U/ml) served as controls and the CA 125 levels were measured before and after addition of 500 ml/l murine serum (Table 1). The levels were only insignificantly affected.

DISCUSSION

False high results have been described for proteins and peptides measured by immunometric assays [2–4]. The false results may be explained by non-analytic substances capable of linking the labelled antibody to the antibody on the solid phase. In the CA 125 assay the same murine, monoclonal antibody is used as detector and as solid phase antibody and the interfering substance might be human antibodies directed against murine immunoglobulins (heterophilic antibodies). If so, the interaction of heterophilic antibodies and murine immunoglobulins should be blocked by the addition of murine serum [4]. According to this assumption a marked decrease in the CA 125 immunoreactivity was observed in the present study after addition of murine serum to six samples with false high antigen values. Addition of 63 ml/l murine serum was sufficient to detect all the false positive samples. In three serum samples from patients with ovarian cancer the CA 125 content (406, 277 and 10 U/ml) was unaffected by the addition of murine serum.

In a recent investigation of a two-site immunoradiometric assay designed to measure human choriogonadotropin, Bascato and Stuart [5] demonstrated the interfering substance to be IgG antibodies directed against an epitope on the $F(ab')_2$ fragment of IgG common to a variety of animal species. The authors pointed out that heterophilic antibodies may result in errors in any technique involving antigen binding to reagent antibody. The interference of heterophilic antibodies is of particular importance if the CA 125 assay is used in screening for ovarian cancer or for the preoperative differential diagnosis of ovarian tumours. However, heterophilic antibodies may also interfere with the use of OC125 for *in vivo* diagnostic or therapeutic purposes.

In conclusion, false positive CA 125 results may be due to human antibodies directed against murine immunoglobulins and are diminished by addition of murine serum. In healthy subjects, false elevated CA 125 levels are of special importance. Addition of murine serum to these samples is recommended and murine serum should be included in the kit reagents as an additive to the labelled detecting antibody.

Addendum—After having finished the present article we have become aware of the following study concerning the same subject: Klug TL, Green PJ, Zurawski VR Jr, Davis HM. Confirmation of a false-positive result in CA 125 immunoradiometric assay caused by human anti-idiotypic immunoglobulin. *Clin Chem* 1988, **34**, 1071–1076.

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REFERENCES

1. Bast RC Jr, Klug TL, John ES *et al.* A radioimmunoasay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med* 1983, **309**, 883–887.

2. Hunter WM, Budd PS. Circulating antibodies of ovine and bovine immunoglobulin in healthy subjects: a hazard for immunoassays. *Lancet* 1980, **ii**, 1136.
3. Boscato LM, Stuart MC. Incidence and specificity of interference in two-site immunoassays. *Clin Chem* 1986, **32**, 1491–1495.
4. Thompson RJ, Jackson AP, Langlois N. Circulating antibodies to mouse monoclonal immunoglobulins in normal subjects—incidence, species specificity, and effects on a two-site assay for creatine kinase-MB isoenzyme. *Clin Chem* 1986, **32**, 476–481.
5. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988, **34**, 27–33.