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# Interference in Immunoassays by Human Anti-mouse Antibodies

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Mogensen and Moller [1] described interference in the Abbott CA 125 enzyme immunoassay (EIA) by putative human anti-mouse antibodies (HAMA) and demonstrated that this interference could be reduced by diluting the specimen with mouse serum. HAMA are predominantly IgGs that arise in response to immunizing doses of mouse monoclonal antibodies (Mabs) [2, 3]. The high titres often found in HAMA sera should be distinguished from the low levels of anti-mouse activity resulting from heterophile antibodies, mostly IgM [4–8]. Since HAMA are polyclonal and of complex specificity, many different types of interference can be observed in immunoassays with HAMA-containing sera. The type of interference is dictated by the assay configuration (i.e. competitive vs. sandwich, one-step vs. two-step), the antibodies used (specificity, isotype and use as capture or probe reagent), the analyte and the subclass or isotype of the Mab used to immunize the patient.

On injection of a patient with a Mab, HAMA are elicited that typically exhibit both anti-isotype and anti-idiotype specificities. The anti-isotype component may well bind to Mabs in an immunoassay and interfere, especially when the injected Mab and the Mabs used in the assay are of the same isotype. Elevated carcinoembryonic antigen (CEA) values were obtained in a patient who had been injected with mouse Mab B72.3 upon assay with the Abbott list 5863 product, which is a double monoclonal sandwich EIA in a two-step format (Table 1). That these increased values resulted from positive interference by HAMA was shown by reassay after removal of HAMA by either heat treatment or chromatography on immobilized protein A (patient 1, Table 2). Similar positive interferences in immunoassays have been reported for other commercially available immunoassays [2, 3, 9, 10]. These false positives, and presumably those described by Mogensen and Moller [1], result from HAMA bridging the probe and capture antibodies and were only partly corrected by diluting the specimen with mouse serum.

Sandwich assay formats that we have evaluated and found to be most resistant to HAMA effects are double polyclonal assays or monoclonal/polyclonal assays, in which the Mab is used as probe. Our CEA EIA One-Step (list 4439), which uses guinea-pig anti-CEA as capture antibody and a Mab as probe, yielded an appropriate value on the high titre HAMA specimen (Table 1). Efficient recovery of CEA (103%) added to this specimen demonstrated that false negatives were not produced.

HAMA may also produce false negatives. For example, in immunoassays with mouse Mabs as capture antibodies and probes that are not mouse Mabs, HAMA binds to the solid phase Mab and sterically blocks capture of the antigen from the specimen, but does not recognize the probe. The addition of mouse serum to HAMA-containing specimens decreases but does not always eliminate this interference.

*Table 1. CEA assay performance in presence of HAMA.*

| Serum date | CEA 5863 (ng/ml) | CEA 4439 (ng/ml) |
|---|---|---|
| 15/3/88 (pretreatment) | 1.83 | 1.74 |
| 5/4/88 | 44.2 | 0.23 |
| 13/5/88 | 53.2 | 0.53 |
| 19/5/88 | >60 | 0.25 |
| 8/6/88 | >60 | 0.17 |
| 15/6/88 | >60 | 0.26 |

Specimen was from patient 1 with ovarian cancer treated with Mab B72.3, a murine IgG1,k. HAMA response was characterized as anti-isotype.

The anti-idiotype component of HAMA may interfere in an immunoassay. This subpopulation of HAMA is specific for the immunizing antibody and thus becomes significant only when that antibody is also used in the assay. For example, patients who have been injected with OC 125 to detect or treat ovarian cancer typically mount a significant anti-idiotype response (patient 2, Table 2). Since the CA 125 radioimmunoassay and EIA use OC 125 both as capture and probe antibodies, falsely high CA 125 values were observed on specimens from these patients due to HAMA bridging capture and probe Mab. This anti-idiotype component has no effect on other immunoassays and is not titred out by diluting the specimen with irrelevant mouse IgG, which lacks the specific OC 125 antigenic determinants.

The clinician has to decide, often on limited immunological data, as to whether immunoassay results obtained with a mouse Mab-based assay in the presence of HAMA are correct or not. Until specific immunoassays can be designed or shown to be refractory to HAMA, no mouse Mab immunoassay should be used in a HAMA-containing specimen. We include this advice in package inserts of Abbott diagnostic kits at risk for this interference (e.g. CA 125 EIA) and are developing methods to eliminate HAMA interference in immunoassays.

*Table 2. Assay results from HAMA containing specimens*

| | | | | Stripped† | |
|---|---|---|---|---|---|
| Patient* | CEA 5863 (ng/ml) | CEA 4439 (ng/ml) | CA 125 RIA | Serum CA 125 (U/ml) | Serum CEA(5863) (ng/ml) |
| Patient 1 | | | | 10 | <2 |
| Pre-treatment | 1.83 | 1.74 | 10 | 10 | 0 |
| 13/5 | 53.2 | 0.53 | 1000 | 55 | 0 |
| Patient 2 | 0 | 0.32 | 8492 | | |

*Patient 1 had anti-isotype response (titre 25,000) on 13/5; patient 2 had anti-idiotype response (titre 2000).
†Serum was stripped of human and mouse IgG by passing over protein A affinity column; circulating tumour markers CEA, TAG72 and CA125 do not bind to protein A.
RIA = radioimmunoassay.

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# Hepatocellular Carcinoma Associated with Other Primary Malignancies

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THERE ARE several case reports about hepatocellular carcinoma (HCC) associated with second and even third primary malignancies [1] and two larger series [2, 3]. We report the occurrence of other primary malignancies in a series of 179 cases of HCC diagnosed in our unit from June 1981 to November 1989.

The diagnosis of HCC was suggested by ultrasound and confirmed by ultrasonically guided fine-needle biopsy [4] in 80% of cases and by laparoscopically controlled biopsy in 9%. In the remaining patients the diagnosis was made on the basis of ultrasonically detected focal liver lesions and by measurement of alpha-fetoprotein (AFP; Enzygnost-AFP): normal below 12 ng/ml, diagnostic above 500 ng/ml. In these patients impaired coagulation contraindicated biopsy.

Seventeen of the 179 patients with HCC (9.5%) had other cancers. One had two other primary malignancies: laryngeal cancer diagnosed 108 months before HCC and non-Hodgkin's lymphoma (NHL) simultaneously diagnosed. In the other 16 patients we found: NHL (3) and colon (2), rectal (1), breast (3), lung (1), gastric (2), uterine (1) and prostatic (2) cancers. One patient had Bowen's disease. In 14 of these 16 the discovery of the associated malignancy preceded that of HCC by several months (mean 46, range 3–120). In one patient the diagnoses were simultaneous and in another patient the diagnosis was made 22 months after that of HCC. The clinical and diagnostic features of these 17 cases of HCC were similar to those found in a larger series of HCC [5]. The pathological findings (evaluated in 15 cases) were: well differentiated HCC cells in seven cases, poorly differentiated cells in seven and pleomorphic large cells in one. The mean age at the time of diagnosis of HCC was 67 years (range 51–78). The male/female ratio was 1.8:1. Hepatic cirrhosis was present in 15 out of 17 patients. Only one patient was HBsAg positive and another six had HBs and/or HBe and/or HBc antibodies. AFP was diagnostic in seven patients.

The patient with two other tumours had been operated on for laryngeal cancer and was treated with chemotherapy for NHL. Four out of 16 patients with a tumour in addition to HCC had undergone chemotherapy for the associated cancer, five surgical removal, three surgery plus chemotherapy and one surgery plus radiotherapy. Two patients received hormonal therapy.

In the large Japanese series of 417 autopsy cases of HCC [6], 32 had associated cancers; three had multiple cancer. Associated cancers occurred in 5.8% of cases in 1967–74 and 8.3% in 1974–81. Cancers of the gastrointestinal tract comprised 56%. Only one case was associated with malignant lymphoma. Three cases had triple cancer. In the Hungarian series [2], nine out of 47 patients with HCC (19%) had an associated cancer (one of these had two other cancers). The most frequently associated tumour was renal cell carcinoma (three cases). One case of NHL was reported. Lin *et al.* from Taiwan [3], which is an area highly endemic for HCC, reported that 12 out of 562 patients (2.1%) had another malignancy. The most common second neoplasm was gastric cancer (eight cases). This tumour is the third most common neoplasm among men in Taiwan. However, the frequency in this series was so high that Lin *et al.* suggested a common environmental or genetic factor as promoter for HCC and gastric cancer. Only one patient had NHL.

In our series HCC was associated with a second malignancy in 9.5% of cases. The most commonly associated second neoplasm was NHL. HCC was the most frequently associated cancer in our previous series of 156 cases of NHL [7]. The association of HCC with NHL that we find probably reflects ascertainment bias in our institution. However, environmental or genetic factors could be causes.

Most patients developed HCC after another tumour and about half received chemotherapy. Could such drugs be carcinogenetic in the liver? Methotrexate is such a drug [8] but this agent was not used in our patients. Familial polyposis coli, which has neoplastic potential throughout the gastrointestinal tract [9], has been associated with HCC. HCC in this condition, in the absence of any other risk factor, has been considered a manifestation of the oncogenicity of the disease [10]. Familial polyposis coli was not found in our three patients out of five with gastrointestinal cancers who had colonoscopy.

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