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Transient human anti-mouse antibodies (HAMA) interference in CA 125 measurements during monitoring of ovarian cancer patients treated with murine monoclonal antibody

Angèle L.M. Oei [a,*], Fred C.G.J. Sweep [b], Leon F.A.G. Massuger [a], André J. Olthaar [b], Chris M.G. Thomas [a,b]

[a] Department of Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
[b] Department of Chemical Endocrinology, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

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Abstract

Objective. To investigate the influence of human anti-mouse antibodies (HAMA) on serial CA 125 measurements in serum of patients with epithelial ovarian cancer following single intraperitoneal (IP) therapy with Yttrium-90-labeled human milk fat globule 1 murine monoclonal antibody (^{90}Y-muHMFG1) as part of a large international randomized phase III trial.

Methods. We monitored CA 125 concentrations in longitudinally collected serum samples from 224 patients after IP ^{90}Y-muHMFG1 (study group) and from 223 patients who received standard treatment (control group). Serum samples of 22 study patients with increased CA 125 concentrations were selected and subjected to affinity chromatography to study HAMA interference in CA 125 measurements.

Results. CA 125 serum concentrations at weeks 1, 4 and 8 were significantly higher in the study group than in the control group. In the first 8 weeks after IP ^{90}Y-muHMFG1 administration significantly more patients of the study group (144/224) demonstrated CA 125 concentrations above the upper limit of normal of 23 U/mL, as compared to those of the control group (37/223). Affinity chromatography of serum with high CA 125 values in the first 8 weeks confirmed HAMA interference in CA 125 measurements while after 24 weeks this HAMA interference could no longer be detected.

Conclusions. This is the first study to demonstrate that clinical trials applying murine monoclonal antibodies may be flawed by a transient HAMA effect, which should be considered when monitoring ovarian cancer patients with CA 125 measurements.

Keywords: CA 125; Monoclonal antibodies; HAMA; Ovarian cancer

Introduction

There has been extensive experience with the serum tumor marker CA 125 in the detection and management of ovarian cancer. Up until now this tumor marker is the best available marker for monitoring epithelial ovarian cancer [1]. Rising values of CA 125 during follow up of ovarian cancer patients correlates with disease progression in approximately 90% of the cases and persistent CA 125 elevation correlates with persistent disease [2].

The prognosis of ovarian cancer is still poor and in need of new treatment modalities [3]. Radioimmunotherapy and immunotherapy which use monoclonal antibodies (Mab) could be such modalities. The murine human milk fat globule 1 (muHMFG1) directed towards the MUC1 antigen, which is overexpressed in 90% of the epithelial ovarian cancers, is a Mab used in clinical trials in ovarian cancer patients [4]. The injection of this mouse Mab induces the development of human anti-mouse antibodies (HAMA) [5]. HAMA are reported to interfere in CA 125 measurements [6–11].

Abbreviations: FPLC, Fast Protein Liquid Chromatography; HAMA, human anti-mouse antibodies; IP, intraperitoneal; Mab, monoclonal antibody; ND, not detectable; TSH, thyroid stimulating hormone.

* Corresponding author. Fax: +31 24 3668597.
E-mail address: A.Oei@obgyn.umcn.nl (A.L.M. Oei).

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In the present study, serial CA 125 measurements of ovarian cancer patients participating in a large international randomized phase III trial [12] treated with a single intraperitoneal (IP) yttrium-90-labeled murine HMFG1 (^{90}Y-muHMFG1) injection (study group) were compared with serial CA 125 measurements of patients who received standard treatment (control group). In addition, we investigated whether differences in CA 125 concentrations existed and if so, to what extent these were caused by the development of HAMA.

Materials and methods

Patients' eligibility

In an international phase III trial [12], comprising 74 institutes, ovarian cancer patients in complete clinical remission FIGO stage Ic-IV were randomly assigned to standard consolidation treatment plus IP ^{90}Y-muHMFG1 (study group) and standard treatment alone (control group). Patients with a macroscopically negative second-look laparoscopy were eligible to receive a single IP dose of 25 mg of the radiolabeled Mab (maximum dose 1110 MBq) or standard treatment between 4 and 8 weeks after receiving their final cycle of chemotherapy. Patients with HAMA serum concentrations (ELISA, Roche Diagnostics, Basel, Switzerland) greater than 50 ng/mL at the start of the study were excluded. The study was approved by the scientific and ethical authorities of the participating institutes and was conducted in compliance with the standards of the Declaration of Helsinki. Serum samples for CA 125 measurements of the control and the study group were taken on the following time points: prior to IP ^{90}Y-muHMFG1 administration (week 0), and at weeks 1, 4 and 8 after ^{90}Y-muHMFG1 administration and at three-monthly intervals up to the closure of the study. To study HAMA interference in CA 125 measurements we tested serum samples of 22 study patients.

CA 125 measurements were performed with the commercially available CA 125 assay (AxSYM, Abbott Laboratories, Chicago, Illinois, USA) with a minimum detectable concentration of 2 U/mL. This assay is based on Microparticle Enzyme Immunoassay technology and uses the murine monoclonal antibody OC 125 as catching antibody. An upper limit of normal CA 125 values of 23 U/mL was used [13].

HAMA measurements were performed using a commercially available ELISA (Roche Diagnostics, Basel, Switzerland) in which HAMA is bound to mouse-IgG-biotin and to the peroxidase-conjugated detection antibody. This complex binds via the biotin-labeled antibody to the streptavidin-coated surface of the microplate. Following the washing step, complex bound peroxidase (HRP) reacts with the substrate tetramethylbenzidine and the product is quantified at 450 nm. The limit of detection is 1.5 ng/mL anti-mouse IgG.

Chromatography procedure

To determine whether the developed HAMA interfere in the CA 125 assay, affinity chromatography (Fast Protein Liquid Chromatography, FPLC) was performed on a number of serum samples with high CA 125 values as described earlier [8]. Patient serum (250 μL) was applied to a HiTrap Protein G column (GE Healthcare, Uppsala, Sweden). The total passage fraction was collected using a 0.02 M phosphate buffer pH 7.0 at a flow rate of 0.5 mL/min. The Protein G-bound IgG was eluted with 1 ml of 0.1 M glycine-HCl buffer, pH 2.7. Directly after elution the pH was neutralized using 50 μL of 1 M Tris–HCl, pH 9.0. The isolated HAMA-IgG in the 4.2-fold diluted eluent fraction was quantified with the CA 125 AxSYM assay and expressed in terms of apparent CA 125 concentrations. CA 125 concentration in the passage fraction was also measured. Due to the 4.2-fold sample dilution of both fractions, CA 125 concentrations could be quantified with a minimum detectable concentration of 8.4 U/mL. In each chromatography run we processed a positive control sample with a known concentration of CA 125. Moreover, we have tested CA 125 values in 3 serum samples obtained at week 8 of the control group of the present study. These control samples always contained ≥80% of the CA 125 measured before chromatography in the effluent fraction (i.e passing the column) of the column and CA 125 values measured in the eluate fractions (eluted from the column) were invariably below the detection limit of the CA 125 assay.

Statistical analysis

Differences in patient characteristics and CA 125 profiles were tested with the Mann Whitney U test with the level of significance set at <0.05. Correlations between HAMA and CA 125 levels during follow up were analyzed with Spearman's correlation test.

Results

The study group consisted of 224 patients who received IP ^{90}Y-muHMFG1 and the control group comprised 223 patients only receiving standard treatment. Prior to Mab administration none of the patients had HAMA levels above 50 ng/mL. Following IP ^{90}Y-muHMFG1 administration, 98.2% of the study group patients developed HAMA levels above 50 ng/mL. Only four patients failed to develop a HAMA response (<50 ng/mL). In the control group none of the patients developed a HAMA response.

Prior to IP ^{90}Y-muHMFG1 administration at week 0, there was no significant difference ($P=0.29$) between CA 125 values of the study ($n=224$) and the control group ($n=223$). At weeks 1, 4 and 8 after ^{90}Y-muHMFG1 administration CA 125 values of the study group were significantly ($P<0.05$) higher as compared to the control group. The difference in CA 125 values between the study and the control group could no longer be observed at 24 weeks and later following IP ^{90}Y-muHMFG1 administration. Significantly more patients of the study group (144/224) had CA 125 values of >23 U/mL (Fig. 1) during the first 8 weeks as compared to the control group (37/223). None of the control patients had CA 125 values greater than twice the upper limit of normal CA 125 values (i.e. ≥46 U/mL) on 2 consecutive time points during the first 8 weeks, while 41 patients of the study group showed CA 125 values of ≥46 U/mL.

To study whether elevated serum CA 125 values in the study group were caused by HAMA interference in the assay, serum samples of these patients were tested for the presence of HAMA by affinity chromatography. The results are shown in Table 1. Serum samples of patients with high CA 125 values during the first 8 weeks and between week 12 and the end of follow-up

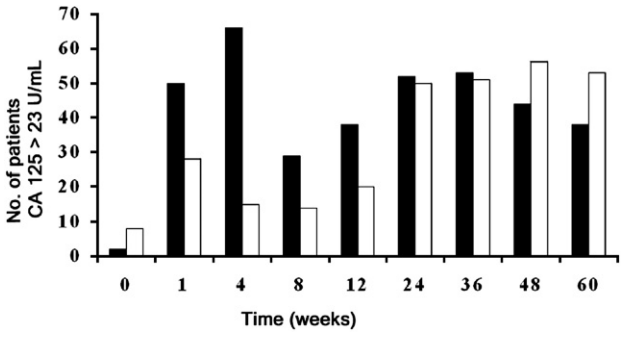

Fig. 1. Number of patients who developed CA 125 values above the upper limit of normal (>23 U/mL) during the first 60 weeks. More patients in the study group ($n=224$, ■) developed CA 125 values of >23 U/mL as compared to the control group ($n=223$, □).