

Determination of MUC1 in Sera of Ovarian Cancer Patients and in Sera of Patients with Benign Changes of the Ovaries with CA15-3, CA27.29, and PankoMab

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Abstract. Aim: Mucin 1 (MUC1) is a high molecular weight transmembrane glycoprotein with unique properties which is used as a tumour marker in sera of ovarian cancer patients. The common test kit for the cancer antigen 15-3 (CA15-3) is not sufficient for the discrimination between sera from healthy individuals and sera from patients with benign changes of the ovaries. In this study, the newly developed anti-MUC1 antibody PankoMab was tested in normal and patient sera with an ELISA, and the obtained data were compared against data from experiments using the commercial kits for CA15-3 and CA27.29. Materials and Methods: Sera of 123 patients diagnosed with benign or malignant changes of the ovaries were obtained before surgery. CA15-3 was analysed with an automated ELISA system (Immulite 2000). CA27.29 was measured with the ST AIA-PACK CA27.29 for the AIA-600II-Analyzer (Tosoh Bioscience, Belgium). The release of MUC1 fragments carrying the TA-MUC1 epitope was analysed with an ELISA using the PankoMab antibody. Results: Using the already established markers CA15-3 and CA27.29, significant differences between benign and malignant changes of the ovaries were found. The same result was obtained with the newly developed TA-MUC1 test. In contrast to CA15-3 and CA27.29, however, the median of TA-MUC1 was lower in sera from patients with ovarian cancer compared to sera from patients with benign diseases of the ovary. However, sera of patients with benign ovarian diseases had significantly higher TA-MUC1 values compared to sera of healthy individuals.

The risk score of TA-MUC1 achieved an area under the curve (AUC) of 78.4% in receiver operating characteristic (ROC) curves and a sensitivity of 37% for the prediction of ovarian disease, at 95% specificity. Conclusion: In this study we employed an additional marker for MUC1 which recognizes a more tumour-specific MUC1 epitope (TA-MUC1). We obtained results showing significant differences between detection in benign and malignant ovarian diseases. Although the mean MUC1 values were elevated in sera of patients with ovarian cancer compared to values of patients with benign cysts, by using all three test systems, a different result was found by analysing the median TA-MUC1 values. PankoMab could be a useful, additional tool for obtaining conclusive information on the transformation process from benign to malignant state in ovarian tissues.

Ovarian cancer consists of many subtypes and represents the fourth most frequent type of cancer among females. It is the leading cause of death from gynaecological cancer in the Western world (1-4). Besides the histopathological subtype, the grading, the clinical staging and the amount of residual tumour, a number of additional putative prognostic tumour markers have been suggested for monitoring this disease (5, 6).

Epithelial mucin 1 (MUC1) is a high molecular weight transmembrane glycoprotein expressed at the apical (luminal) membrane of many types of normal epithelial cells (7). On malignant cells, an increased and often depolarized expression (including basolateral cell membranes and the cytoplasm) has been recorded (8).

Overexpression of MUC1 disrupts cell-cell and cell-matrix adhesions (9-11). It is generally accepted that the MUC1 overexpression by the tumour cells facilitates the invasive growth and the metastasis (12, 13). In addition, MUC1 has been shown to contribute to cancer cell escape from immune surveillance, as MUC1-expressing cells are less susceptible to T-cell- and natural killer (NK) cell-mediated lysis (14).

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Soluble MUC1 (also known as CA15-3) is a routinely employed tumour marker in the clinic for the diagnosis and the disease management of invasive breast cancer (15). Overproduction of MUC1 in ovarian cancer also leads to increased circulating levels of this molecule, detectable by standardised tests (CA15-3 and/or CA27.29, respectively). CA15-3 is elevated in approximately 70% of patients with epithelial ovarian cancer, predominantly in those with advanced disease (16, 17). The overall survival rate for this group of patients is very low. On the other hand, 90% of women diagnosed with disease confined to the ovary, survive more than five years (18). Therefore, the early detection of ovarian cancer is crucial.

The development of serum-based diagnostic kits for the detection of early-stage ovarian cancer is an urgent need. Since new diagnostic markers of ovarian cancer might be useful for screening purposes, we tested the newly developed anti-MUC1 antibody PankoMab in an ELISA assay. PankoMab (19) is a novel MUC1 antibody explicitly tailored to recognize the tumour-associated MUC1 epitope (TA-MUC1), as described in earlier studies (20). In a recent study, we demonstrated that PankoMab's may also be suited as a diagnostic antibody in breast cancer. PankoMab reactivity revealed a strong correlation with the expression of the estrogen receptor (ER) (21). Therefore the aim of this study was to test PankoMab as a potential serum tumour marker for the detection of early forms of ovarian cancer by the determination of TA-MUC1 in sera of healthy controls and in sera of patients with benign or malignant ovarian disease.

Materials and Methods

Sera. Sera from 123 patients diagnosed with either benign (83) or malignant (40) disease of the ovaries were obtained before surgery. After surgery, histological diagnostic evaluation and staging of the tumour was performed by an experienced gynaecological pathologist (D.M.) according to the criteria of the International Federation of Gynaecologists and Obstetricians (FIGO) and the World Health Organization (WHO).

CA15-3 ELISA. The concentration of CA15-3 was determined by an ELISA using an Immulite 2000 automated diagnostic system (Siemens, Munich, Germany). The standard deviation for precision at 9 U/ml is 0.44 with a variation coefficient (CV) of 4.9%. Precision analysis showed no cross reactivity with human alpha fetoprotein (AFP), CA19-9, cisplatin, cyclophosphamide, CA125, carcinoma embryonic antigen (CEA), doxorubicin, 5-fluorouracil, mitomycin C, and vincristine.

CA27.29 ELISA. The concentrations of CA 27.29 were measured with the ST AIA-Pack 27.29 reagent using an AIA-600II-Analyzer (Tosoh Bioscience, Tessenderlo, Belgium) according to the manufacturer's instructions. The ST AIA-Pack 27.29 assay is an automated monoclonal fluorometric assay directed against the MUC-1 antigen. An amount of 150 µl of serum was diluted at 1:20, and the monoclonal antibody, already bound to magnetic beads, was

added. The beads were washed and incubated at 37°C with the fluorogenic substrate 4MUP. Assay results above 31 U/ml were regarded as positive. In all positive samples, two repeats of the assay were performed, and the mean was calculated.

PankoMab ELISA. The release of MUC1 fragments carrying the TA-MUC1 epitope was analysed with an ELISA using the PankoMab antibody (Glycotope, Berlin, Germany).

Statistical analysis. Statistical analysis was performed using SPSS 18.0 (PASW Statistic, SPSS Inc., IBM, Chicago, IL, USA). Correlation analysis of the MUC1 release was performed using the non-parametric Kruskal Wallis rank-sum test, the non-parametric Mann-Whiney *U*-test, and receiver operating characteristic (ROC) analysis. *p*-values below 0.05 were considered statistically significant.

Results

CA15-3 ELISA. We identified significant differences in CA15-3 values between sera of patients with benign ovarian cysts or tumours, compared to those with malignant carcinomas as analysed with the Mann-Whitney *U*-test ($p=0.007$). Patients with malignant tumours had significantly higher serum CA15-3 levels compared to patients with benign tumours. The median CA15-3 level was 46 U/ml for sera of patients with malignant disease, and 21 U/ml for sera of patients with benign disease (Figure 1).

CA27.29 ELISA. Sera from 76 controls without benign or malignant disease were analysed, with none of the samples having values above 31 U/ml. We measured significant differences of CA27.29 values between normal sera, sera of patients with benign ovarian tumours, and sera from patients with carcinomas as calculated with the Kruskal Wallis rank-sum test ($p<0.001$). Patients with malignant tumours had higher serum CA27.29 levels compared to patients with benign tumours. The median CA27.29 level was 37 U/ml for malignant diseases, 16 U/ml for benign diseases, and 10.9 U/ml for control sera (Figure 2).

PankoMab ELISA. We observed significant differences in the TA-MUC1 levels analysed with PankoMab in control sera ($N=31$), in sera from patients with benign ovarian cysts or tumours, and in sera from patients with carcinomas, according to the Kruskal Wallis rank-sum test ($p<0.001$). The median level for TA-MUC1 was 22 U/ml in sera of patients with ovarian carcinoma compared to 31 U/ml in the sera of patients with benign disease, and 12 U/ml for control sera (Figure 3).

ROC analyses. The ROC curves for patients with benign ovarian cysts or tumours compared to the ones of healthy individuals are presented in Figure 4, and the AUC were calculated. The resulting sensitivities for the prediction of benign ovarian cysts or tumours compared to normal controls were 37% at 95% specificity for PankoMab, and 48% at 95% specificity for CA27.29, and the AUC were 78.4% for PankoMab and 82.7 % for CA27.29.

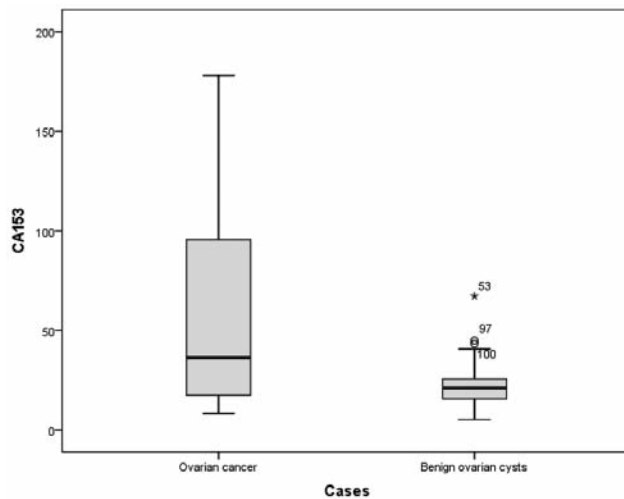


Figure 1. Determination of CA15-3 levels in sera of patients diagnosed with benign or malignant diseases of the ovaries before surgery, shown in box plots. The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. Bars delineate the 5th and 95th percentiles. Circles indicate values more than 1.5× the box length, and asterisks values more than 3.0× the box length from the 75th percentile. CA 15-3 levels were significantly lower in patients with benign diseases compared to patients with malignant diseases of the ovaries ($p=0.007$).

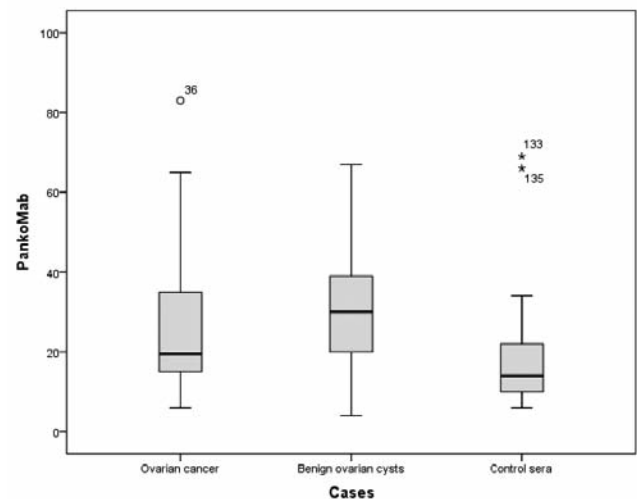


Figure 3. Determination of TA-MUC1 levels (analysed with PankoMab ELISA) in healthy control sera, and in sera of patients diagnosed with benign or malignant diseases of the ovaries, before surgery. Values are shown in box plots. The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. The bars delineate the 5th and 95th percentiles. Circles indicate values more than 1.5× the box length, and asterisks more than 3.0× box the length from the 75th percentile. TA-MUC1 levels were significantly higher in patients with benign diseases compared to patients with malignant diseases of the ovaries, as well as compared to healthy controls ($p<0.001$).

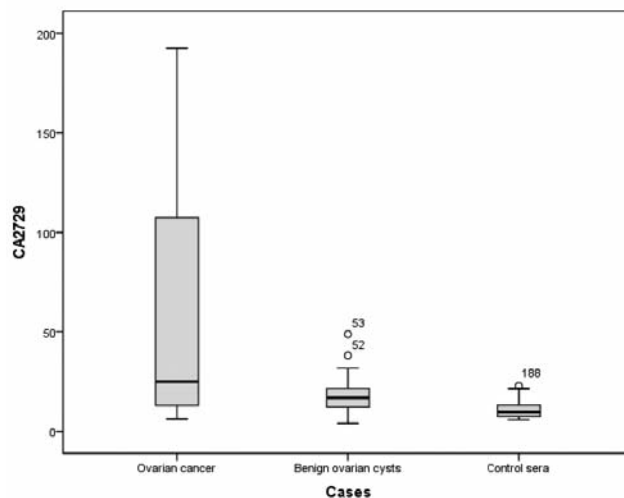


Figure 2. Determination of CA27.29 levels in healthy control sera and in sera of patients diagnosed with benign or malignant diseases of the ovaries, before surgery. Values are shown in box plots. The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. Bars delineate the 5th and 95th percentiles. Circles indicate values more than 1.5 × the box length, and asterisks values more than 3.0× the box length from the 75th percentile. CA 27.29 levels were significantly higher in patients with malignant compared to patients with benign diseases of the ovaries, as well as compared to healthy controls ($p<0.001$).

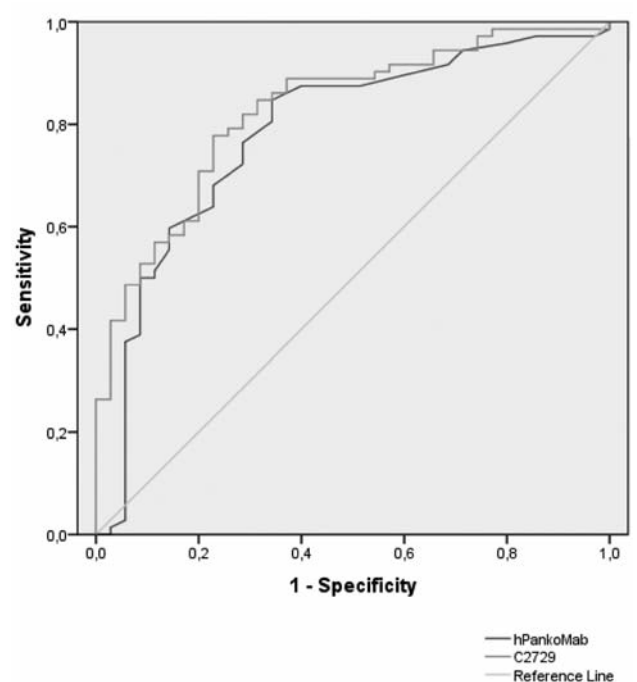


Figure 4. Receiver operating characteristic curves indicating the profile of sensitivity and specificity for the estimation of benign diseases of the ovaries and of healthy controls using the CA27.29 and PankoMab tests.

Discussion

In this study, we tested TA-MUC1 (measured with PankoMab) as a potential serum marker for the detection of early forms of ovarian cancer. PankoMab (19) is a novel MUC1 antibody explicitly tailored to recognize a tumour-associated MUC1 epitope (TA-MUC1) as described earlier (20). This epitope consists of a special carbohydrate-induced conformation of the PDTRP motif (22). PankoMab has improved tumour selectivity, making it very attractive as a potential therapeutic antibody (19). When we examined serum MUC1 with three antibodies, the established CA15-3 and CA27.29 systems and the new PankoMab antibody, we observed that the median of TA-MUC1 was – in contrast to CA15-3 and CA27.29, values-lower in the sera of patients with ovarian cancer compared with sera from patients with benign diseases. This is an interesting fact relating well to data previously reported for the sera of patients with colon and pancreatic cancer [19], and indicating that PankoMab as a therapeutic antibody would be less bound to shed MUC1 than other antibodies. One may speculate that in serum from individuals with cancer, the conformation of the TA-MUC1 epitope is unstable. However, TA-MUC1 as measured with PankoMab might be suitable as a serum marker for the detection of benign ovarian diseases. The difference was especially pronounced when comparing the median TA-MUC1 values. The risk score of TA-MUC1 achieved an AUC of 78.4% in ROC curves, and the sensitivity for the prediction of ovarian disease was 37% at 95% specificity. Therefore, PankoMab could be a useful additional tool for obtaining conclusive information for the transformation process from benign to malignant status in ovarian tissues (19, 21, 23).

In a recent study, we demonstrated that PankoMab may be suitable as a diagnostic antibody in breast cancer immunohistochemistry. In contrast to the anti-MUC1 antibodies DF3 and VU-4-H5, PankoMab's reactivity revealed a strong correlation with the expression of the ER (21). No correlation to lymph node involvement was found.

In conclusion, PankoMab is a novel anti-MUC1 antibody with diagnostic potential providing strong correlation in sera from patients with benign ovarian diseases *versus* normal sera, a property that was not found with in the established anti-MUC1 tumour markers CA15-3 or CA27.29 used for comparison.

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