EUROPEAN JOURNAL OF CANCER 43 (2007) 1556–1563

available at www.sciencedirect.com

journal homepage: www.ejconline.com

Humoral immune responses to MUC1 in women with a *BRCA1* or *BRCA2* mutation

B.B.J. Hermsen[a,*], *R.H.M. Verheijen*[a], *F.H. Menko*[b], *J.J.P. Gille*[b], *K. van Uffelen*[c], *M.A. Blankenstein*[c], *S. Meijer*[d], *P.J. van Diest*[e], *P. Kenemans*[a], *S. von Mensdorff-Pouilly*[a]

[a]*Department of Obstetrics and Gynaecology, VU University Medical Centre, De Boelenlaan 1117, 1081 HV Amsterdam, The Netherlands*
[b]*Department of Clinical Genetics and Human Genetics, VU University Medical Centre, The Netherlands*
[c]*Department of Clinical Chemistry, VU University Medical Centre, The Netherlands*
[d]*Department of Surgical Oncology, VU University Medical Centre, The Netherlands*
[e]*Department of Pathology, University Medical Centre, Utrecht, The Netherlands*

ARTICLE INFO

Article history:
Received 22 December 2006
Received in revised form 6 March 2007
Accepted 2 April 2007
Available online 25 May 2007

Keywords:
BRCA1/2 mutations
MUC1
Natural antibodies

ABSTRACT

Introduction: Breast cancer patients with early disease and a natural humoral response to MUC1 have a favourable prognosis, suggesting a possible role of MUC1 antibodies (ab) in controlling haematogenous tumour dissemination and outgrowth. The aim of the study was to evaluate humoral immune responses to MUC1 in women at hereditary high risk of breast cancer to investigate whether this immune response could play a role in the prevention of disease.

Materials and methods: CA15.3 (U/mL), and IgG and IgM ab to MUC1 (arbitrary units per mL, Arb-U/mL) were measured in serum samples obtained from 422 women at hereditary high risk of breast/ovarian cancer, of whom 127 *BRCA1/2* carriers, attending the Familial Cancer Clinic of the VU University Medical Centre, and from 370 age-matched healthy controls. Serum samples obtained from women who developed breast cancer ($N = 12$) or breast cancer recurrence ($N = 17$), and from women who underwent prophylactic mastectomy ($N = 12$) and had no breast lesions were also tested.

Results: CA15.3 ranked significantly higher in mutation carriers than in controls ($P = 0.03$). MUC1 IgG ab levels ranked significantly lower in *BRCA1/2* mutation carriers than in controls ($P = 0.003$). MUC1 IgG levels were not significantly different ($P = 0.53$) between women who developed primary breast cancer (median 0.72 Arb-U/ml, range 0.52–2.44 Arb-U/ml) and women who underwent prophylactic mastectomy and had no breast lesions (median 1.04 Arb-U/ml, range 0.43–2.88 Arb-U/ml).

Conclusion: Serum levels of natural IgG ab to MUC1 are lower in *BRCA1/2* mutation carriers than in healthy controls. Furthermore, in contrast to previous results in women with sporadic breast cancer, no elevated MUC1 IgG ab were seen in women at hereditary high risk who developed breast cancer. Prophylactic immunotherapy with MUC1 substrates may be a strategy to reduce the risk of breast cancer in *BRCA1/2* mutation carriers, strengthening tumour immune surveillance.

* *Corresponding author*: Tel.: +31 20 4444851; fax: +31 20 4443333.
E-mail address: b.hermsen@vumc.nl (B.B.J. Hermsen).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.04.007

1. Introduction

Interest in the detection of a germline mutation in the *BRCA1*[1] or *BRCA2*[2] gene is based on its potential to identify women with a substantial high risk of breast and ovarian cancer that would benefit from preventive measures. Estimates of breast and ovarian cancer risk in women carrying a *BRCA1/2* mutation by 70 years of age range from 56% to 80% for breast cancer, and from 16% to 40% for ovarian cancer.[3–5]

These women at high risk of breast and ovarian cancer are included in screening programmes directed towards early detection of disease[6–8] or they undergo prophylactic surgery. Due to the limitations of screening, the most effective preventive measure for women at high risk is prophylactic mastectomy (pM) and/or prophylactic bilateral salpingo-oophorectomy (pBSO). The latter has been shown to reduce not only the risk of ovarian cancer[9] but also that of breast cancer.[10,11]

These radical methods still suffer from some limitations as pBSO does not protect from primary peritoneal carcinomatosis,[12] and a 5% residual risk of breast cancer is still present after pM. This limited prevention efficacy should be looked at in the light of the mutilating effect of pM, and the hormonal deprivation and subsequent morbidity caused by pBSO.[13]

Other forms of preventive treatment are therefore warranted, such as prophylactic vaccination to create an adequate immune surveillance against adenocarcinomas.[14] Cancer vaccines are being developed for the treatment of cancer but also with an eye on prevention,[15] and some common tumours may be prevented by specific vaccines administered to patients with a preneoplastic lesion or with a genetic risk of developing cancer.[16] A strong immune response induced by active immunotherapy may provide a constant surveillance mechanism that would protect women with a genetically determined high cancer risk from developing breast and/or ovarian cancer.

MUC1, a cell surface antigen expressed in glandular epithelia, is overexpressed and aberrantly glycosylated in adenocarcinomas. The peptides and glycans of MUC1 are being studied as substrates for cancer vaccines.[17] A natural humoral immune response to MUC1 has been associated with a favourable disease outcome in patients with breast, lung and pancreatic cancer.[18–20] We observed that women with breast cancer with early disease and a natural humoral response to MUC1 have a relatively low chance of developing metastases and a favourable prognosis, suggesting a possible role of MUC1 antibodies in controlling haematogenous tumour dissemination and outgrowth.[18]

In the present study, we analysed serum levels of MUC1 (CA15.3) and natural antibodies to MUC1 in women at hereditary high risk of breast /ovarian cancer compared to controls. Secondly, we investigated within the group of women at hereditary high risk whether the levels of natural antibodies to MUC1 differed between women who developed breast cancer and healthy women who underwent pM.

2. Material and methods

2.1. Study population

The study population consisted of 422 women at hereditary high risk for breast and/or ovarian cancer undergoing gynaecological and surgical screening as part of the cancer preventive strategy at the Familial Cancer Clinic of the VU University Medical Centre, between 1993 and 2002. Table 1 lists the clinical characteristics of the women at first visit. Women who had had no menstrual period for 12 months or more were defined as post-menopausal; otherwise, they were classified as premenopausal.

Pedigree analysis was performed for each individual. A clinical diagnosis of hereditary breast/ovarian cancer syndrome (HBOC) was assigned to those individuals belonging to a family with three or more cases of breast and/or ovarian cancer present in two generations, and a clinical diagnosis of familial breast/ovarian cancer syndrome (FBOC) to those individuals belonging to a family with two cases of breast and/or ovarian cancer.

DNA assessment was performed after counselling and informed consent. One hundred and twenty five women (30%) declined DNA assessment, leaving 297 women (70%) in whom *BRCA1/2* mutations were detected by a combination of protein truncation test (PTT), denaturing gradient gel electrophoresis (DGGE) and direct DNA sequencing (DS).[21] The large exons 11 of *BRCA1* and 10 and 11 of *BRCA2* were screened by PTT; the remaining exons of both genes were screened by DGGE. If aberrations were detected, DNA sequencing was used to elucidate the exact mutation. Additionally, we performed multiplex ligation-dependent probe amplification (MLPA) to screen *BRCA1* or exon deletions or duplication.[22] *BRCA1/2* mutations were detected in 127 women (30%), results were inconclusive in 137 women (32%), and 33 women were proven non-mutation carriers (8%). The latter were excluded from further evaluation. The control population consisted of 370 healthy women with a median age of 43 years (range 20–70 years). Controls were age-matched to the hereditary high risk women out of a cohort of 938 healthy women abstracted from a cohort of women described earlier.[23,24] As no information on the menstrual history of these women was available, we defined women from 52 years and older as post-menopausal ($N = 68$), and otherwise as premenopausal ($N = 302$).

The research protocol was approved by the Scientific Committee of the VU University Medical Centre Research Institute for Cancer and Immunology.

2.2. Serum samples

Serum samples were collected serially at every visit to the Familial Cancer Clinic, aliquoted and stored at −70 °C until analysed. We tested serum samples obtained from 370 controls and from 389 women at hereditary high risk at first visit to the Familial Cancer Clinic. Furthermore, we analysed serum samples obtained from women who developed a primary breast cancer ($N = 12$) or a breast cancer recurrence ($N = 17$) during screening, and from healthy women who underwent prophylactic mastectomy and had no breast lesions ($N = 12$). All serum samples were obtained within 12 months of surgery. The median time between obtaining the serum samples and date of breast cancer diagnosis, date of recurrence and date of prophylactic mastectomy was 8 months (range 0–9 months), 4 months (range 0–10 months) and 5 months (range 0–12 months), respectively.

Table 1 – Characteristics of the total study population at first visit

	Women at hereditary high risk			
	Carrier, N = 127 (%)	High-risk and no mutation detected, N = 137 (%)	High-risk and no DNA assessment, N = 125 (%)	Total, N = 389 (%)
Median age (years)	40	43	41	41
(Range)	(20–70)	(21–65)	(23–69)	(21–70)
Clinical diagnosis				
Hereditary (HBOC)	123 (97)	76 (55)	78 (62)	277 (71)
Familial (FBOC)	4 (3)	61 (45)	47 (38)	112 (29)
Mutation analysis				
BRCA1 mutation	98 (77)	0	0	98 (77)
BRCA2 mutation	29 (23)	0	0	29 (23)
History of breast cancer				
No	80 (63)	77 (56)	107 (86)	264 (68)
Yes	47 (37)	60 (44)	18 (14)	125 (32)
Parity				
Nulliparous	41 (32)	32 (23)	40 (32)	113 (29)
Multiparous	86 (68)	105 (77)	85 (68)	276 (71)
Menopausal status				
Pre-menopausal	97 (76)	107 (78)	100 (80)	304 (78)
Post-menopausal	30 (24)	30 (22)	25 (20)	85 (22)

Abbreviations: NA = not applicable, Nulliparous = no full term pregnancy or delivery, Multiparous = one or more full term pregnancy or deliveries.

2.3. *MUC1 antibody assay*

Circulating antibodies to MUC1 were measured with an enzyme-linked immunoassay as previously described.[25] In short, 60-mer peptide (corresponding to three tandem repeats of the MUC1 peptide core and conjugated to bovine serum albumin [BSA]) and BSA were adsorbed in alternate rows in 96-well enzyme-linked immunoadsorbent assay (ELISA) plates. After overnight incubation the wells were incubated with BSA to block non-specific adsorption sites. Serum samples diluted 1:100 (for IgG determinations) and 1:500 (for IgM determinations) were incubated overnight and immunoglobulins bound to the peptide were detected with horseradish peroxidase-conjugated rabbit anti-human IgG or IgM (DAKO A/S, Glostrup, Denmark), diluted 1:10.000. Tetramethylbenzidine (TMB) was used as substrate and the reaction was quantified at 450 nm in an ELISA reader. Each serum sample was tested separately for the presence of IgG and IgM MUC1 antibodies. The assay was performed in duplicate for each serum sample and the results were calculated as the mean difference between the readings in optical density units (OD) in experimental wells and controls. A four point standard curve was made for each plate using huHMFG-1 (Antisoma, London, UK), a humanised IgG ab to MUC1, for the IgG determinations, and 2F8 (Dr. S. Kaul, Heidelberg, Germany), a human IgM ab to MUC1, for the IgM determinations. An arbitrary unit of '1' was ascribed to the lowest concentration and the level of reactivity of the samples tested was calculated within each plate in relation to the standard curve by least square regression analysis and expressed in arbitrary units per mL (Arb-U/mL).

2.4. *CA15.3 assay*

CA15.3 was measured with the ADVIA Centaur CA15.3 assay according to the instructions provided by the manufacturer (Bayer Diagnostics Mijdrecht, The Netherlands). The assay is a two-step sandwich immunoassay using direct, chemiluminescent technology. The lite reagent is composed of the monoclonal mouse antibody DF3, specific for CA15.3, labeled with acridinium ester. The conjugate reagent is composed of the monoclonal mouse antibody 115D8, specific for CA15.3, labeled with fluorescein. The solid phase is composed of purified monoclonal mouse capture antibody, which is covalently coupled to paramagnetic particles. Sensitivity and assay range is 0.50–200 U/ml. The inter-assay coefficient of variation (CV) at 20 U/ml, 50 U/ml and 190 U/ml was 4%, 4% and 3%, respectively. The cut-off level of 30 U/ml (95 percentile of a normal population) used for the analysis was that provided by the manufacturer.

2.5. *Statistical analysis*

Statistical analysis was performed using SPSS software (Version 11.5, SPSS Inc, Chicago, IL). Results were analysed for normality of distribution. CA15.3 serum levels and MUC1 antibody assay results in the different groups were analysed using the Mann–Whitney U/Wilcoxon rank sum W test. The correlation among assay results and the correlation between age and IgG antibody levels against MUC1 were evaluated by linear regression analysis. Two-sided testing was applied and the significance level was set at $\leqslant 0.05$.

3. Results

3.1. Antibodies to MUC1

IgM antibodies to MUC1 did not differ between women at hereditary high risk and controls (results not shown). MUC1 IgG antibody levels and corresponding CA15.3 levels are listed in Table 2 and Table 3. Neither MUC1 IgG nor CA15.3 varied significantly with age in women at hereditary high risk or in controls ($P = 0.21$ and $P = 0.47$, respectively). The 95 percentile of MUC1 IgG antibody levels in the high-risk population and controls was 3.68 Arb-U/ml and 2.07 Arb-U/ml, respectively. MUC1 IgG antibody levels, however, ranked significantly lower ($P = 0.01$) in women at hereditary high risk (median 0.76 Arb-U/ml, range 0.32–19.16 Arb-U/ml) than in controls (median 0.86 Arb-U/ml, range 0.43–12.60 Arb-U/ml).

Furthermore, MUC1 IgG antibody level ranked significantly lower in women with a HBOC syndrome than in controls ($P = 0.003$), but did not differ between women with a FBOC syndrome and controls ($P = 0.48$). MUC1 IgG levels in mutation carriers ranked significantly lower than in controls ($P = 0.003$), see Fig. 1. No difference was seen in MUC1 IgG levels between women with an inconclusive DNA result ($P = 0.82$) or women with no DNA assessment ($P = 0.32$) and controls. MUC1 IgG levels did not differ between *BRCA1* and *BRCA2* carriers ($P = 0.08$).

Additionally, we investigated if a personal history of breast cancer could be of influence on the MUC1 IgG antibody level in the high-risk group. From the 389 women at hereditary high risk, 125 (32%) had a personal history of breast cancer. No difference ($P = 0.69$) in MUC1 IgG levels was found between women with a history of breast cancer (median 0.74 Arb-U/ml, range 0.40–19.16 Arb-U/ml) and women with no history of breast cancer (median 0.77 Arb-U/ml, range 0.32–14.98 Arb-U/ml). Furthermore, MUC1 IgG antibody levels did not differ ($P = 0.60$) between *BRCA1/2* mutation carriers with or without a history of breast cancer.

No significant differences were seen in MUC1 IgG levels in relation to parity or pre-menopausal status. However, in post-menopausal women at high risk, MUC1 IgG levels (median 0.74 Arb-U/ml, range 0.40–19.16 Arb-U/ml) ranked significantly lower ($P = 0.006$) than in post-menopausal controls (median 0.95 Arb-U/ml, range 0.47–12.60 Arb-U/ml). Both the post-menopausal women at hereditary high risk with no mutation found and the post-menopausal *BRCA1/2* carriers had significantly lower levels of IgG levels than post-menopausal controls ($P = 0.04$ and $P = 0.007$, respectively).

Table 2 – Median (range) MUC1 IgG antibodies (Arb-U/ml) in relation to clinical characteristics

	Women at hereditary high risk, N = 389				Controls, N = 370
	Mutation carrier, N = 127	High-risk and no mutation detected, N = 137	High-risk and no DNA assessment, N = 125	Total, N = 389	
MUC1 IgG ab (Arb-U/ml)	0.74 (0.32–19.16)	0.76 (0.37–16.78)	0.77 (0.36–14.98)	0.76 (0.32–19.16)	0.86 (0.43–12.60)
Clinical diagnosis					
Hereditary (HBOC)	0.74 (0.39–19.16)	0.74 (0.37–8.81)	0.76 (0.36–14.98)	0.75 (0.36–19.16)	NA
Familial (FBOC)	0.47 (0.32–1.16)	0.83 (0.43–16.78)	0.83 (0.39–8.03)	0.82 (0.32 –16.78)	
Mutation analysis					
BRCA1 mutation	0.76 (0.43–10.48)	NR	NR	0.76 (0.43–10.48)	NA
BRCA2 mutation	0.64 (0.32–19.16)			0.64 (0.32–19.16)	
History of breast cancer					
No	0.74 (0.32–7.35)	0.83 (0.37–10.09)	0.76 (0.36–14.98)	0.77 (0.32–14.98)	NA
Yes	0.70 (0.40–19.16)	0.74 (0.41–16.78)	0.79 (0.52–5.44)	0.74 (0.40 –19.16)	
Parity					
Nulliparous	0.70 (0.32–10.48)	0.73 (0.37–8.81)	0.93 (0.44–8.03)	0.75 (0.32–10.48)	NA
Multiparous	0.74 (0.39–19.16)	0.80 (0.37–16.78)	0.75 (0.36–14.98)	0.76 (0.36–19.16)	
Menopausal status					
Pre-menopausal	0.78 (0.32–4.94)	0.76 (0.37–8.81)	0.78 (0.36–8.03)	0.77 (0.32–8.81)	0.84 (0.43–8.28)
Post-menopausal	0.65 (0.40–19.16)	0.77 (0.49–16.78)	0.75 (0.42–14.98)	0.74 (0.40–19.16)	0.95 (0.47–12.60)

NR, not relevant; NA, not applicable.

Table 3 – Median (range) of the tumour marker CA15.3 (U/ml) in relation to clinical characteristics

	Women at hereditary high risk, N = 389				Controls, N = 370
	Mutation carrier, N = 127	High-risk and no mutation detected, N = 137	High-risk and no DNA assessment, N = 125	Total, N = 389	
CA15.3 (U/ml)	16 (3–37)	14 (2–46)	12 (1–39)	13 (1–46)	13 (3–58)
Clinical diagnosis					
Hereditary (HBOC)	16 (3–37)	14 (4–46)	13 (1–39)	14 (1–46)	NA
Familial (FBOC)	12 (5–31)	13 (2–32)	10 (3–29)	12 (2–32)	
Mutation analysis					
BRCA1 mutation	16 (4–37)	NR	NR	16 (4–37)	NA
BRCA2 mutation	14 (3–37)			14 (3–37)	
History of breast cancer					
No	16 (3–36)	12 (4–46)	12 (2–39)	13 (2–46)	NA
Yes	16 (5–37)	15 (2–31)	12 (4–25)	15 (2–37)	
Parity					
Nulliparous	16 (4–37)	14 (4–30)	9 (1–35)	13 (1–37)	NA
Multiparous	15 (3–37)	13 (2–46)	12 (2–39)	13 (2–46)	
Menopausal status					
Pre-menopausal	15 (3–37)	13 (2–31)	12 (1–39)	13 (1–39)	13 (3–58)
Post-menopausal	20 (5–37)	18 (3–46)	13 (2–28)	18 (2–46)	16 (5–39)

NR, not relevant, NA, not applicable.

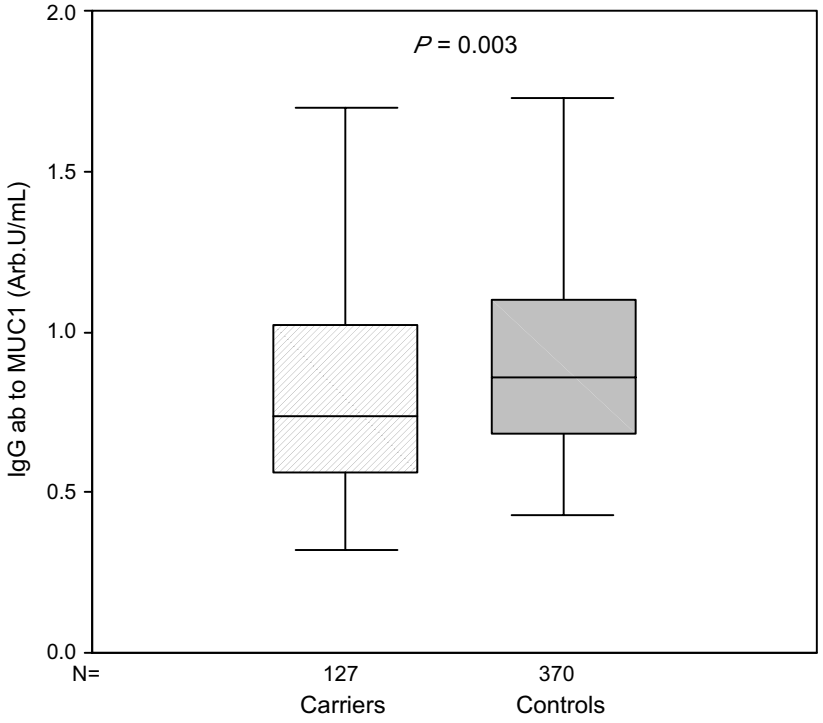

Fig. 1 – Box-and-whisker plot of MUC1 IgG levels in BRCA1/2 carriers and controls. The upper and lower borders of the boxes represent the 75th and 25th percentiles, respectively. The horizontal lines in the boxes are medians. MUC1 IgG levels ranked significantly lower in carriers than in controls.

3.2. CA 15.3

The 95 percentile of the tumour marker CA15.3 was 28 U/ml and 26 U/ml for women at hereditary high risk and controls, respectively. At first visit, CA15.3 was above the 30 U/ml cut-off level recommended by the assay manufacturer in 12 (3%) women at hereditary high risk (median 35 U/ml, range 31–46 U/ml), and in 8 (2%) controls (median 32 U/ml, range 31–

58 U/ml). One woman with an elevated CA15.3 was diagnosed with pT1N0M0 breast cancer, and 3 women had a history of breast cancer.

CA15.3 levels were similar in women at hereditary high risk (median 13 U/ml, range 1–46 U/ml) and controls (median 13 U/ml, range 3–58 U/ml). In women with a HBOC syndrome, CA15.3 levels (median 14 U/ml, range 1–46 U/ml) ranked significantly higher ($P = 0.01$) than in women with a FBOC syndrome (median 12 U/ml, range 2–32 U/ml). Equally, in mutation carriers CA15.3 ranked significantly higher than in controls ($P = 0.03$). Additionally, CA15.3 ranked higher ($P = 0.05$) in women with a previous history of breast cancer (median 15 U/ml, range 2–37 U/ml) versus women with no history of breast cancer (median 13 U/ml, range 2–46 U/ml).

No differences in CA15.3 levels were found according to parity and menopausal status, with one exception: CA15.3 levels were higher in nulliparous carriers than in the other nulliparous high-risk women ($P = 0.009$).

3.3. *MUC1 IgG and CA15.3 and breast cancer*

Marker levels were analysed in women at hereditary high risk who developed a primary breast cancer in the course of screening ($N = 12$, including 7 *BRCA1/2* carriers) or who developed a recurrence of breast cancer ($N = 17$, including 6 *BRCA1/2* carriers) and they were compared to marker levels in women at hereditary high risk with no personal history of breast cancer who underwent prophylactic mastectomy and had no (pre) malignant lesions in their removed breasts ($N = 12$, all but one *BRCA1/2* carrier). Twelve tumours were detected during breast cancer screening at the Family Cancer Clinic; three women were diagnosed with a DCIS, 3 women had stage I, and 6 women had stage II disease. A breast cancer recurrence was diagnosed in 7 women with stage I, 8 women with stage II and 2 women with stage III disease. MUC1 IgG levels were not significantly different ($P = 0.53$) between women who developed primary breast cancer (median 0.72 Arb-U/ml, range

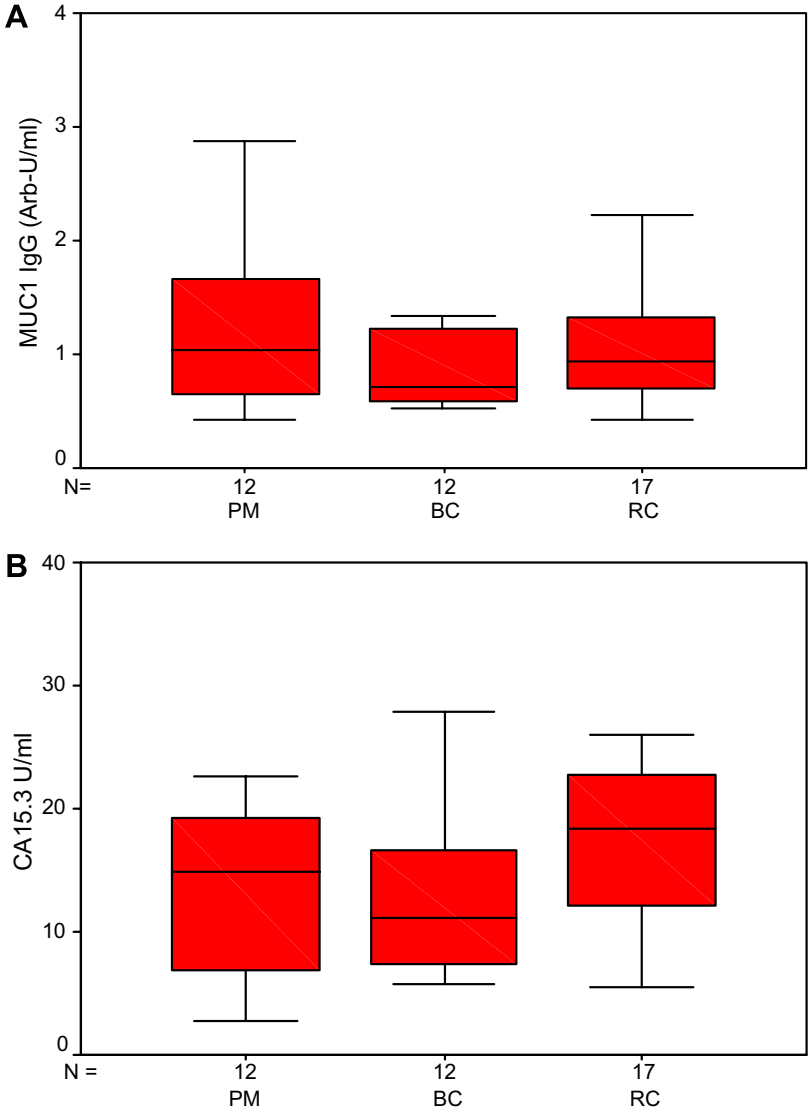

Fig. 2 – Box-and-whisker plot of MUC1 IgG levels (A) and CA15.3 levels (B) in women who underwent surgery for prophylactic mastectomy (PM), or breast cancer (BC) or had a recurrence of breast cancer (RC). The upper and lower borders of the boxes represent the 75th and 25th percentiles, respectively. The horizontal lines in the boxes are medians. MUC1 IgG and CA15.3 levels did not differ significantly between these three groups of women.

0.52–2.44 Arb-U/ml) and women who underwent prophylactic mastectomy and had no breast lesions (median 1.04 Arb-U/ml, range 0.43–2.88 Arb-U/ml) (Fig. 2A).

4. Discussion

To our knowledge, this is the first study investigating MUC1 IgG antibody levels in women at hereditary high risk for breast cancer. We found that *BRCA1/2* mutation carriers have a significantly lower level of MUC1 IgG antibodies than healthy controls. Furthermore, in accordance to our earlier results,[18] we expected that women at hereditary high risk who developed breast cancer would also show an increase in MUC1 IgG antibody levels as do women with sporadic breast cancer. However, MUC1 IgG levels did not differ between women with or without a personal history of breast cancer, suggesting a diminished ability to respond to the antigen in women with hereditary high risk to breast/ovarian cancer. This inability to generate an immune response to an antigen abundantly expressed on breast cancer tumour cells may contribute towards the early onset of breast cancer in women at hereditary high risk.

Median CA15.3 was within normal levels, but ranked significantly higher ($P = 0.03$) in *BRCA1/2* mutation carriers than in controls, whereas MUC1 IgG ab levels ranked significantly lower ($P = 0.003$). It is unlikely that MUC1 IgG ab were spuriously low due to interference with CA15.3 as this would only have occurred if the latter levels were low in the mutation carriers instead of high. The fact that CA15.3 is higher in the mutation carriers could be due to the reported higher incidence of premalignant lesions in mutation carriers.[26]

Little is known about a possible relation between *BRCA1/2* mutation and the immune system. Healthy women at hereditary high risk of breast/ovarian cancer have no clinical signs of an immune deficiency. Nevertheless, a recent study reported that germline mutations of *BRCA1* are associated with a selective deficiency in spontaneous and LPS-induced production of TNF-α and of TNF-α-induced ICAM-1 expression on peripheral blood monocytes.[27] ICAM-1 (CD54) acts as a costimulatory molecule necessary for the emergence of an appropriate immune response via interaction between antigen presenting cells and T cells.[28] TNF-α is of biologic importance as a triggering signal for a series of control mechanisms regulating the development of malignancy. Thus, the TNF-α-dependent defect in upregulation of ICAM-1 on monocytes derived from healthy *BRCA1* germline mutation carriers could result in a series of impairments of the immune function, obviously compensated for by the regular baseline expression of this molecule. Intradermal skin tests to assess for delayed type hypersensitivity (DTH) could be carried out to further explore immune competence in women at high risk.

Women with early stage sporadic breast cancer and an immune response to MUC1 show a benefit in survival,[18] suggesting that MUC1-based immunotherapy could positively influence outcome of disease. If immunotherapy is most effective in the early stages of tumour growth, immunoprevention should be considered. Induction of appropriate immunosurveillance by means of vaccination with MUC1 glycopeptides may constitute a simple and low-invasive preventive measure for high-risk women. The use of immunological measures to prevent or delay the onset of cancer in healthy persons has not received much attention. Specific antitumour vaccination directed to induce immune responses to MUC1, a tumour antigen abundantly expressed in adenocarcinomas of the breast, ovary and Fallopian tube, is a possible approach to this end. Furthermore, premalignant lesions in breast, ovary and Fallopian tube have been identified in women with a *BRCA1/2* mutation,[29] suggesting a good target for specific vaccination.

A detailed analysis of serum natural IgG antibodies to different glycoforms of the MUC1 mucin may contribute towards identifying the best immunotherapeutic approach with MUC1. Serum from breast cancer patients recognise more effectively different glycoforms of MUC1 than the naked MUC1 peptide, indicating that glycosylation of the peptide may be critical for immunorecognition, and therefore for immunotherapeutic approaches.[30] Binding patterns of murine monoclonal antibodies to the immunodominant region of MUC1 are conditioned by glycosylation. Improved binding to the immunodominant motif glycosylated at the T of the PDTR was seen with monoclonal antibodies generated against tumour-derived MUC1.[31] An evaluation of immune responses to MUC1 using glycopeptides such as those used in previous studies,[30,32] or novel glycoforms of MUC1, such as those containing the Sialyl-Tn cancer-associated antigen,[33] may be a good strategy for defining the best MUC1 glycopeptide for immunotherapy. Furthermore, the detailed analysis of MUC1 glycoforms expressed in premalignant lesions and breast carcinomas of women at hereditary high risk, using specific monoclonal antibodies,[31,34] could contribute towards identifying the best MUC1 glycoforms for immunotherapy.

In conclusion, serum levels of natural IgG antibodies to MUC1 were significantly lower in *BRCA1/2* mutation carriers than in healthy controls. Furthermore, in contrast to previous results in women with sporadic breast cancer, women at hereditary high risk that developed breast cancer did not show an increase of MUC1 IgG antibodies. Therefore, prophylactic immunotherapy with MUC1 substrates may be a strategy to reduce the risk of breast/ovarian cancer in *BRCA1/2* mutation carriers, strengthening tumour immune surveillance. A possible relation between *BRCA1/2* mutations and the immune system remains to be explored. Well-designed clinical trials of prophylactic vaccinations for women at hereditary high risk for breast and/or ovarian cancer should be strongly considered, particularly given the current practice of prophylactic bilateral mastectomy and salpingo-oophorectomy.

Conflict of interest statement

None stated.

Acknowledgements

This work was supported in part by a grant from the Biocare Foundation (Grant No. 2002- 22). We thank Ms. A. Kok for expert technical assistance.

REFERENCES

1. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;**266**(5182):66–71.
2. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1995;**378**(6559):789–92.
3. Ford D, Easton DF, Peto J. Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. *Am J Hum Genet* 1995;**57**(6):1457–62.
4. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med* 1997;**336**(20):1401–8.
5. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science* 2003;**302**(5645):643–6.
6. Bourne TH, Campbell S, Reynolds KM, et al. Screening for early familial ovarian cancer with transvaginal ultrasonography and colour blood flow imaging. *BMJ* 1993;**306**(6884):1025–9.
7. Vasen HF, Haites NE, Evans DG, et al. Current policies for surveillance and management in women at risk of breast and ovarian cancer: a survey among 16 European family cancer clinics. European Familial Breast Cancer Collaborative Group. *Eur J Cancer* 1998;**34**(12):1922–6.
8. Sauven P. Guidelines for the management of women at increased familial risk of breast cancer. *Eur J Cancer* 2004;**40**(5):653–65.
9. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. *N Engl J Med* 2002;**346**(21):1609–15.
10. Hartmann LC, Schaid DJ, Woods JE, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. *N Engl J Med* 1999;**340**(2):77–84.
11. Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. *N Engl J Med* 2002;**346**(21):1616–22.
12. Tobacman JK, Greene MH, Tucker MA, Costa J, Kase R, Fraumeni JF. Intra-abdominal carcinomatosis after prophylactic oophorectomy in ovarian-cancer-prone families. *Lancet* 1982;**2**(8302):795–7.
13. Madalinska JB, Hollenstein J, Bleiker E, et al. Quality-of-life effects of prophylactic salpingo-oophorectomy versus gynecologic screening among women at increased risk of hereditary ovarian cancer. *J Clin Oncol* 2005;**23**(28):6890–8.
14. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 2004;**21**(2):137–48.
15. Finn OJ, Forni G. Prophylactic cancer vaccines. *Curr Opin Immunol* 2002;**14**(2):172–7.
16. Lollini PL, Forni G. Antitumor vaccines: is it possible to prevent a tumor? *Cancer Immunol Immunother* 2002;**51**(8):409–16.
17. Taylor-Papadimitriou J, Burchell JM, Plunkett T, et al. MUC1 and the immunobiology of cancer. *J Mammary Gland Biol Neoplasia* 2002;**7**(2):209–21.
18. Mensdorff-Pouilly S, Verstraeten AA, Kenemans P, et al. Survival in early breast cancer patients is favorably influenced by a natural humoral immune response to polymorphic epithelial mucin. *J Clin Oncol* 2000;**18**(3):574–83.
19. Hirasawa Y, Kohno N, Yokoyama A, Kondo K, Hiwada K, Miyake M. Natural autoantibody to MUC1 is a prognostic indicator for non-small cell lung cancer. *Am J Respir Crit Care Med* 2000;**161**:589–94.
20. Hamanaka Y, Suehiro Y, Fukui M, Shikichi K, Imai K, Hinoda Y. Circulating anti-MUC1 IgG antibodies as a favorable prognostic factor for pancreatic cancer. *Int J Cancer* 2003;**103**(1):97–100.
21. Sevilla C, Moatti JP, Julian-Reynier C, et al. Testing for BRCA1 mutations: a cost-effectiveness analysis. *Eur J Hum Genet* 2002;**10**(10):599–606.
22. Hogervorst FBL, Nederlof PM, Gille JJP, et al. Large genomic deletions and duplications in the BRCA1 gene identified by a Novel Quantitative Method. *Cancer Res* 2003;**63**(7):1449–53.
23. Bon GG, Kenemans P, Verstraeten R, van Kamp GJ, Hilgers J. Serum tumour marker immunoassays in gynecologic oncology: establishment of reference values. *Am J Obstet Gynecol* 1996;**174**:107–14.
24. Kenemans P, Bon GG, Kessler AC, Verstraeten AA, van Kamp GJ. Multicenter technical and clinical evaluation of a fully automated enzyme immunoassay for CA 125. *Clin Chem* 1992;**38**:1466–71.
25. Mensdorff-Pouilly S, Gourevitch MM, Kenemans P, et al. An enzyme-linked immunosorbent assay for the measurement of circulating antibodies to polymorphic epithelial mucin (MUC1). *Tumour Biol* 1998;**19**(3):186–95.
26. Hoogerbrugge N, Bult P, de Widt-Levert LM, et al. High prevalence of premalignant lesions in prophylactically removed breasts from women at hereditary risk for breast cancer. *J Clin Oncol* 2003;**21**(1):41–5.
27. Zielinski CC, Budinsky AC, Wagner TM, et al. Defect of tumour necrosis factor-alpha (TNF-alpha) production and TNF-alpha-induced ICAM-1-expression in BRCA1 mutations carriers. *Breast Cancer Res Treat* 2003;**81**(2):99–105.
28. Wolfram RM, Budinsky AC, Brodowicz T, et al. Defective antigen presentation resulting from impaired expression of costimulatory molecules in breast cancer. *Int J Cancer* 2000;**88**(2):239–44.
29. Hermsen BB, van Diest PJ, Berkhof J, et al. Low prevalence of (pre) malignant lesions in the breast and high prevalence in the ovary and Fallopian tube in women at hereditary high risk of breast and ovarian cancer. *Int J Cancer* 2006;**119**(6):1412–8.
30. von Mensdorff-Pouilly S, Petrakou E, Kenemans P, et al. Reactivity of natural and induced human antibodies to MUC1 mucin with MUC1 peptides and *n*-acetylgalactosamine (GalNAc) peptides. *Int J Cancer* 2000;**86**(5):702–12.
31. Karsten U, Serttas N, Paulsen H, Danielczyk A, Goletz S. Binding patterns of DTR-specific antibodies reveal a glycosylation-conditioned tumour-specific epitope of the epithelial mucin (MUC1). *Glycobiology* 2004;**14**(8):681–92.
32. von Mensdorff-Pouilly S, Kinarsky L, Engelman K, et al. Sequence-variant repeats of MUC1 show higher conformational flexibility, are less densely O-glycosylated and induce differential B lymphocyte responses. *Glycobiology* 2005;**15**(8):735–46.
33. George SK, Schwientek T, Holm B, Reis CA, Clausen H, Kihlberg J. Chemoenzymatic synthesis of sialylated glycopeptides derived from mucins and T-cell stimulating peptides. *J Am Chem Soc* 2001;**123**(45):11117–25.
34. Tarp MA, Sorensen AL, Mandel U, et al. Identification of a novel cancer-specific immunodominant glycopeptide epitope in the MUC1 tandem repeat. *Glycobiology* 2007;**17**(2):197–209.