

## Summary

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Germline mutations in the tumour suppressor genes BRCA1 and BRCA2 predispose women to both breast and ovarian cancer. Although genetic abnormalities occur in all cancers, BRCA mutations are critical as these genes serve to safeguard genetic content. Although protecting genetic information is a general function, BRCA-related pathways seem largely specific to preventing breast and ovarian cancer. Women at hereditary high risk and with a proven BRCA mutation have potentially two options to control their risk of breast and ovarian cancer: either to undergo intensive surveillance or prophylactic surgery, or both.

*The objective of the present thesis (Chapter I) was to investigate a series of potential predictive markers as well as preventive measures presently in use, with the aim to provide a more precise and individual based risk profile for women at hereditary high risk.*

In short:

1. To evaluate the efficacy of gynaecological screening programs for ovarian cancer in women with a proven BRCA1/2 mutation in the Netherlands.
2. To evaluate serum CA125 values in women at hereditary high risk of ovarian cancer and in the presence of (pre)malignant lesions in the adnexa.
3. To investigate the nature of (pre)malignant lesions that occur in prophylactically removed breast and ovarian tissue of patients at hereditary high risk, and analyse whether (pre)malignant lesions are concomitantly present in prophylactically removed breast and adnexal tissue of the same individual.
4. To measure serum levels of natural antibodies to MUC1 in women at hereditary high risk of breast and/or ovarian cancer, to investigate if this immune response could play a role in the prevention of breast and/or ovarian cancer.

In **Chapter II** *the outcomes of gynaecological screening for ovarian cancer* among BRCA1/2 carriers in the Netherlands has been described. Aim of study was to investigate compliance to, and efficacy of, an annual screening in proven BRCA1/2 mutation carriers. Observed numbers of ovarian cancer were compared with expected numbers of cancer. A total of 888 women with a proven BRCA1/2 mutation were included. On average, women were optimally screened for 75% of the total period of follow-up. Compliance decreased with longer follow-up. Five of the ten incident cancers were interval tumours, diagnosed in women who had had a normal screening result within 3-10 months before diagnosis. No difference in stage distribution between incident screen-detected (N=5) and interval tumours (N=5) was found. Eight of the ten incident cancers were stage III/IV (80%). Cancers diagnosed in unscreened family members, had a similar stage distribution (77% in stage III/IV). The observed number of cases detected during screening was not significantly higher than expected (Standardized Incidence Ratio (SIR): 1.5, 95% Confidence Interval: 0.7-2.8).

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For the subgroup that was fully compliant to annual screening, a similar SIR was found (1.6, 95% Confidence Interval: 0.5-3.6). The wide range in the confidence intervals measured suggests that our study population may have been too small to achieve a significant difference (low power).

In **Chapter III** *the value of longitudinal, consecutive measurements of CA125* in a women at hereditary high risk has been described. This study was designed to investigate whether longitudinal increases of CA125 are indicative of the presence of a (pre)malignant lesion in adnexal tissue obtained from women at hereditary high risk of ovarian cancer. We found that CA125 was significantly higher in patients with adnexal dysplasia. Predictive for ovarian cancer was the relative change of CA125 over time. Predictive for dysplasia was the absolute value of CA125. Within the normal range of CA125, women with levels equal to or higher than 14 U/ml had a 48% probability of having dysplasia in the adnexal tissue, but only a 7% probability of having ovarian cancer.

In **Chapter IV** *(pre)malignant lesions and other histopathological findings* present in prophylactically removed breast and adnexal tissue were described. Tissue was obtained from 85 women who underwent prophylactic bilateral salpingo-oophorectomy (pBSO) and from 59 women who underwent prophylactic mastectomy (pM). Control tissue samples were obtained from women undergoing breast reduction surgery (N = 99) or adnexal surgery for benign reasons (N=72). In women with a BRCA1/2 mutation, the prevalence of a (pre)malignant adnexal lesion was 50% (95% CI 26-74) if older than 40 years and 14% (95% CI 0-58) if younger. The prevalences of (pre)malignant breast lesions in women older than 40 years, with and without a BRCA1/2 mutation, were 0% (95% CI 0-16) and 47% (95% CI 21-73), respectively. In 28 women that underwent surgery on both organs no association was found between (pre)malignant lesions in breast and adnexal tissue. The prevalence of lesions was significantly higher in adnexal tissue than in the breast.

*Lobulitis* was a new, unexpected and, moreover, frequent finding in prophylactically removed breast tissue from women at hereditary high risk of breast cancer, and is described in **Chapter V**. Lobulitis was defined by the presence of morphologically normal lobules with more than 100 lymphocytes and/or plasma cells per lobule and in more than one histological section, not in relation to any epithelial lesion and excluding periductitis.

Lobulitis was encountered in 21 out of 41 (51%) patients at high risk; this feature was present in only 8 out of 82 (10%) of the controls ( $P < 0.0001$ ). Lobulitis was equally distributed between proven mutation carriers (14/25) and women at hereditary high risk with no established mutation (7/16). However, the occurrence of lobulitis differed significantly between BRCA1 and BRCA2 mutation carriers ( $P = 0.021$ ). Only one patient out of 7 BRCA2 carriers had lobulitis in comparison with 13 out of 18 BRCA1 carriers. In all cases, the affected lobules showed no atrophy or destruction.

*Characterisation of lymphocytes present in lobulitis* associated to hereditary high risk of breast cancer has been described in the addendum to **Chapter V**. The pattern of the lymphocytic infiltrate did not differ between women at high risk and controls: the infiltrate was composed predominantly of T-cells (DC3). B-cells (CD20) were few and only sporadically present.

Additionally, we investigated the characteristics of intratumoral lymphocytic infiltrates in breast cancer tissue obtained from 7 patients at hereditary high risk. In contrast to lobulitis, macrophages and dendritic cells were present in lymphocytes infiltrating breast cancer tumours suggesting an active immune response against the tumour.

MUC1, a cell surface antigen expressed in glandular epithelia, is overexpressed and aberrantly glycosylated in adenocarcinomas. *A natural humoral immune response to MUC1* has been associated with a favourable disease outcome in patients with breast, lung and pancreatic cancer. Early breast cancer patients with a natural humoral response to MUC1 have a higher probability of freedom from distant metastases and a better disease-specific survival, suggesting a possible role of antibodies against MUC1 in controlling haematogenous tumour dissemination and outgrowth. The peptides and glycans of MUC1 are being studied as substrates for cancer vaccines. In **Chapter VI** the humoral immune response to MUC1 in women with a BRCA1/2 mutation has been described. The rationale behind this investigation was to evaluate humoral immune responses to MUC1 in women at hereditary high risk of breast/ ovarian cancer, because these women are candidates for prophylactic vaccination against cancer. IgG antibodies to MUC1 were measured by ELISA in serum samples obtained at gynaecological screening visits from 422 patients at hereditary high risk and from 370 age-matched healthy controls. MUC1 IgG antibody levels ranked significantly lower in carriers of a BRCA1/2 mutation than in controls. The median IgG level was not significantly different between patients who had a history of breast cancer and no evidence of disease or who developed a recurrence of breast cancer compared to women who had normal breast tissue as found by prophylactic mastectomy.

Based on the results described in this thesis, the following conclusions can be formulated:

1. Efficacy of gynaecological screening for ovarian cancer in women with a proven BRCA1/2 mutation is poor in terms of stage shift. In addition, although the compliance was relatively high, the number of ovarian cancers detected by screening was not significantly higher than the number of malignant tumours detected during an interval visit.
2. A rise in serum CA125 levels can be indicative of dysplasia or carcinoma in ovarian or tubal epithelium.
3. Premalignant lesions are not concomitantly present in prophylactically removed breast and adnexal tissue of the same individual. Premalignant lesions are more frequently present in adnexal tissue than in the breast.
4. Lobulitis is a frequently seen feature in prophylactically removed breast tissue of patients at hereditary high risk of breast cancer, suggesting an early immune response to an as yet unidentified antigen.
5. A lower level of natural antibodies to MUC1 is present in the serum of BRCA1/2 mutation carriers, also in those with a history of breast cancer or a breast cancer recurrence. Stimulation of this immune response with MUC1 vaccines may play a preventive role in women at high risk for breast and/ or ovarian cancer.